PB-227 883

BIOLOGICAL TREATMENT OF PETROCHEMICAL WASTES

R. S. Engelbrecht, et al

Illinois University

Prepared for:

Public Health Service

January 1963

DISTRIBUTED BY:

NTIS

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va. 22151

PB 227 883

BIOLOGICAL TREATMENT OF PETROCHEMICAL WASTES

by

R. S. Engelbrecht, R. E. Speece

and C. V. RamaRao

Final Report

September 1, 1955

through

October 31, 1962

Supported by
Division of Water Supply and Pollution Control
U. S. Public Health Service
Research Project WP-138

Department of Civil Engineering
University of Illinois
Urbana, Illinois

January, 1963

TABLE OF CONTENTS

		Page
List of Tables		iii
List of Figures		iv
Organization of Report		vi
Personnel		vii
Part 1.	Summary of Published Results	1
Part 2.	Treatment of Nitrogen Deficient Wastes by a Modification of the Activated Sludge Process	14
	Abstract	15
I	Introduction	16
II	Literature Survey	19
III	Procedures and Experimental Techniques	30
IV	Results	34
V	Discussion	66
VI	Engineering Significance	82
VII	Conclusions	84
VIII	Bibliography	85
IX	Appendix	87

LIST OF TABLES

Table No.		Page
I	Estimated Nitrogen Savings	78
	Appendix	
I	Warburg Experiments with Glucose	87
II	Warburg Experiments with Phenol	88
III	Warburg Experiments with Acetate	89
IV	Duration of Substrate Removal with no Wasting of Sludge	90
V	Protoplasm Production - Warburg Studies	92

LIST OF FIGURES

	Figure No.		Page
Part 1.			
	1	Diffused Air Stripping of Volatile Compound	5
	2	Effect of Aeration Tank Geometry on the Relationship Between Air Flow Rate and Ka	6
	3	Effect of Temperature on Ka	7
	4	Predicted & Observed Removal of Acetone in Activated Sludge Aeration Tank	9
	5	Removal of Acetone	11
	6	Removal of Butanone	12
Part 2.			
	0	Legend	37
	1	Glucose 500 mg/ℓ COD - F:M 1:1	38
	2	Glucose 500 mg/ℓ COD - F:M 1:2	39
	3	Glucose 1000 mg/ℓ COD - F:M 1:1/2	40
	4	Glucose 1000 mg/ℓ COD - F:M 1:1	41
	5	Glucose 2000 mg/ℓ COD - F:M 1:2	42
	6	Glucose 2000 mg/ℓ COD - F:M 1:1	43
	7	Phenol 500 mg/ℓ COD - F:M 1:1/2	44
	8	Phenol 500 mg/ℓ COD - F:M 1:1	45
	9	Phenol 500 mg/ℓ COD - F:M 1:2	46
	10	Phenol 1000 mg/ℓ COD - F:M 1:1/2	47
	11	Phenol 1000 mg/ℓ COD - F:M 1:1/2	48
	12	Phenol 1000 mg/ℓ COD - F:M 1:1	49
	13	Phenol 1000 mg/ℓ COD - F:M 1:3	50
	14	Phenol 2000 mg/ℓ COD - F:M 1:1/2	51
	15	Acetate 500 mg/ℓ COD - F:M 1:1/2	52
	16	Acetate 500 mg/ℓ COD - F:M 1:1	53
	17	Acetate 500 mg/ℓ COD - F:M 1:2	54

LIST OF FIGURES (Continued)

18	Acetate 1000 mg/ℓ COD - F:M 1:1/2	55
19	Acetate 1000 mg/ℓ COD - F:M 1:1	56
20	Acetate 1000 mg/ℓ COD - F:M 1:2	57
21	Acetate 2000 mg/ℓ COD - F:M 1:1/2	58
22	Duration vs COD Removal Glucose Substrate	59
23	Duration vs COD Removal Phenol Substrate	60
24	Duration vs COD Removal Acetate Substrate	61
25	Glucose 500 mg/ℓ COD - F:M 1:1/4 and 1:1/2 Nitrogen Supplemented	62
26	Glucose 1000 mg/ℓ COD - F:M 1:1/4 Nitrogen Supplemented	63
27	Phenol 500 mg/ℓ COD - F:M 1:1/4 Nitrogen Supplemented	64
28	Acetate 2000 mg/ℓ COD - F:M 1:1/4 Nitrogen Supplemented	65
29	Sludge Production - Glucose	70
30	Sludge Production - Phenol	71
31	Sludge Production - Acetate	72

Organization of Report

In presenting this final report, the results and conclusions of the entire study are reported in two parts. Part I summarizes the results which have been published previously. This material has been extracted from the following publications:

1. "Biological Treatment of Petrochemical Wastes." Progress Report, September 1, 1955 to July 1, 1957. RG-4443. Submitted August 1957.

2. "Biological Treatment of Petrochemical Wastes." Progress Report, July 1, 1957 to January 31, 1958. RG-4443. Submitted February, 1958.

3. Gaudy, A. F., Jr., and Engelbrecht, R. S., "The Stripping of Volatile Compounds." Proceedings of the Fifteenth Industrial Waste Conference, Purdue University, Engineering Extension Series No. 106, 224 (May 1960).

4. Engelbrecht, R. S., Gaudy, A. F., Jr., and Cederstrand, J. M., "Diffused Air Stripping of Volatile Waste Components of Petrochemical Wastes." J. Water Poll. Control Fed., 33, 127 (February 1961).

5. Gaudy, A. F., Jr., Engelbrecht, R. S., and Turner, B. G., "Stripping Kinetics of Volatile Components of Petrochemical Wastes." J. Water Poll. Control Fed., 33, 382 (April 1961).

6. Gaudy, A. F., Turner, B. G., and Pusztaszeri, S., "Biological Treatment of Volatile Waste Components." J. Water Poll. Control Fed., 35, 75 (February 1963).

7. Engelbrecht, R. S., and Ewing, B. B., "Treatment of Petrochemical Wastes by the Activated Sludge Process." Presented at Second Industrial Water and Waste Conference, University of Texas, Austin, Texas, June 1962 (To be published in the conference proceedings and in Industrial Water and Wastes).

Results of the most recent and terminal phase of the overall study are presented in Part 2. This is a report of a study made by Mr. C. V. RamaRao on the treatment of nitrogen deficient wastes, using pure organic compounds, by a modification of the activated sludge process. This report is taken from Mr. RamaRao's thesis which was submitted in February 1963 as partial fulfillment for the Master of Science degree in Sanitary Engineering.

Personnel

Since initiation of the study in September 1955, under the direction of Dr. Jess C. Dietz, a number of personnel changes occurred. In February 1957, Dr. Dietz resigned from the University and, consequently, as Principal Investigator of the study. He was replaced by Dr. R. S. Engelbrecht, who had previously served as a part-time bacteriologist. Dr. A. F. Gaudy became associated with the study in November 1959 as the Co-Principal Investigator with Dr. Engelbrecht. When Dr. Gaudy resigned from the University in September 1961, he was replaced by Dr. R. E. Speece who served as Co-Principal Investigator to the termination of the study. Other personnel of the study, and their period of employment, were as follows:

Mr. D. E. Alton, June 1956 to June 1957

Mrs. J. M. Cederstrand, December 1956 to April 1959

Mr. B. G. Turner, June 1959 to August 1960

Mr. S. Pusztaszeri, July 1960 to February 1962

Mr. P. C. Poon, September 1960 to August 1961

Mr. C. V. RamaRao, September 1961 to September 1962

The contributions made by these persons in carrying out the objectives of this study are sincerely acknowledged.

PART 1

SUMMARY OF PUBLISHED RESULTS

In considering the biological treatment of volatile constituents in petrochemical wastes by the activated sludge process, it was found that such components were subject to stripping by diffused aeration. Thus, the treatment of petrochemical and other wastes which might contain volatile compounds may be accomplished using a physical as well as biological mechanism. This would appear to make the activated sludge process particularly attractive. However, depending upon its relative magnitude, the physical stripping of a portion of waste could be expected to exert a considerable effect upon design loadings and the amount of sludge handled. Further, the stripping of the waste may make it difficult to maintain and renew the requisite biological population. In order to examine biological treatment, it was deemed necessary to first study the factors which affect the stripping phenomena. Pure organic compounds were selected for this phase of the study, primarily because the use of such a model waste enhances experimental control and the general application of conclusions which might be drawn from the research. In any event, there was no representative petrochemical waste which could be used or simulated.

In general, the factors first studied were those which affected stripping kinetics. Volatile compounds in dilute solution may be treated in a manner similar to that used for a gas. Thus, identical kinetic expressions can be used. One expression states that the rate of change in concentration is proportional to the concentration of removable volatile component remaining at any time, t, and to the area available for transfer, A, divided by the total volume of fluid subjected to stripping, V. This is expressed by Equation 1:

$$\log \frac{C_t - S}{C_o - S} = -\frac{K^1}{2.3} \left(\frac{A}{V}\right) t \qquad (1)$$

Since the interfacial area A is subject to change throughout the depth of the tank and is, in any event, extremely difficult to measure, this term is generally included in an apparent or overall k factor, designated ka, which may be applied to the study of a specific system under constant conditions. Since ka contains a variable component, its theoretical significance is lessened. However, it may serve as a convenient basis for comparing the various compounds as to their affinity for stripping. Assuming the residual concentration, S, is small, Equation 1 may be modified to the simple first-order equation expressed by Equation 2:

$$\log \frac{C_t}{C_o} = -kat \qquad (2)$$

The relationship between unit air flow rate and ka can be expressed by Equation 3:

$$ka = C\ Qa^n \qquad (3)$$

where C is a constant depending on the temperature and the specific compound tested and Qa is the unit air flow rate. The value of n for the pure compounds studied was found to be in the order of 0.35. Another form can also be used to express the relation between ka and Qa as shown in Equation 4:

$$ka = ka_o + R\ Qa \qquad (4)$$

A series of batch stripping experiments was conducted to determine the volatilization of a number of selected pure compounds which would very likely be a component in the waste waters from a petrochemical plant. All experiments were continued for eight hours and the over-all transfer coefficient was determined. Experimental conditions were varied and their effect on the transfer coefficient of each compound was observed.

Acetone, propionaldehyde, butanone, butyraldehyde and valeraldehyde were the compounds chosen for study. The results are shown in Figure 1. The removal of acetone, methyl ethyl ketone and propionaldehyde followed first order kinetics at 25°C and a unit air flow rate of 900 cc/min/ℓ (120 ft^3/min/1000 gal). However the removal of butyraldehyde and valeraldehyde at 25°C and propionaldehyde at 40°C did not obey first order kinetics under those conditions.

Further studies on acetone showed that the initial concentration had no effect on the over-all transfer coefficient. Also, the concentration of inorganic salts did not affect the rate of stripping. The effect of aeration tank geometry was studied by using a confined column aerator and a conventional aerator which results in a "rolling action" turbulence. While the tank geometry did affect the absolute value of the transfer coefficient, it did not affect the applicability of the first order function. Figure 2 shows the effect of tank geometry and unit air flow rate on the transfer coefficient. The effect of temperature on the over-all transfer coefficient and the relationship between unit air flow rates and over-all transfer coefficient were also studied. Figure 3 illustrates the temperature effect. Thus, these last three factors, temperature, unit air flow rate, and tank geometry most critically affected stripping.

Next, studies were designed to examine the degree to which substrate removal could be predicted for any given activated sludge system when both physical stripping and biological metabolism were operative. The separate kinetic constants for each removal mechanism were defined independently and then a combined kinetic equation was formulated.

When both the physical stripping and biological removal mechanisms are considered to occur simultaneously, the rate of removal of the pure compound must be the sum of the removal rates afforded by each mechanism. If both mechanisms follow first-order kinetics, Equation 5 can be used to

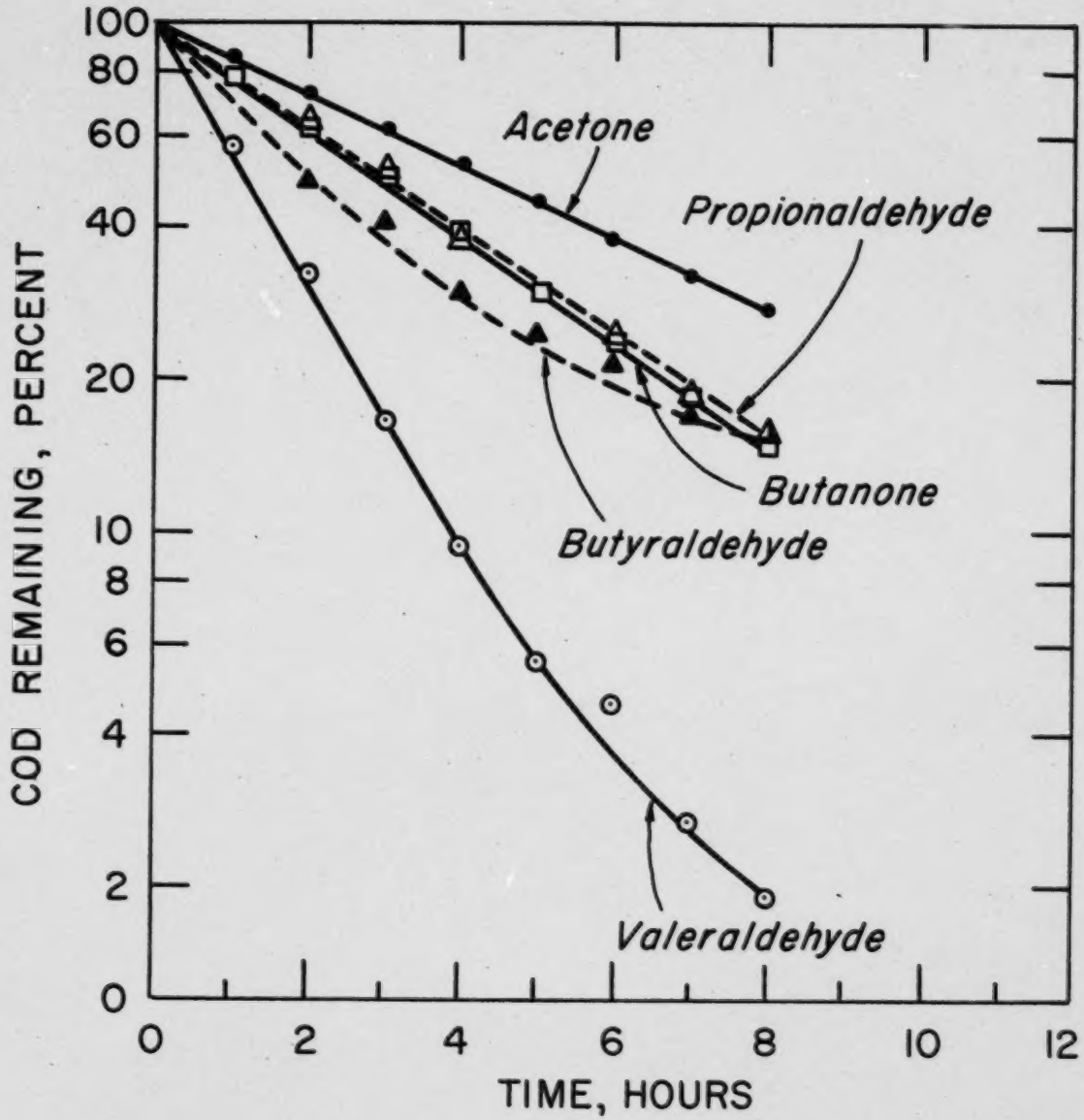


FIGURE 1 - DIFFUSED AIR STRIPPING OF VOLATILE COMPOUND

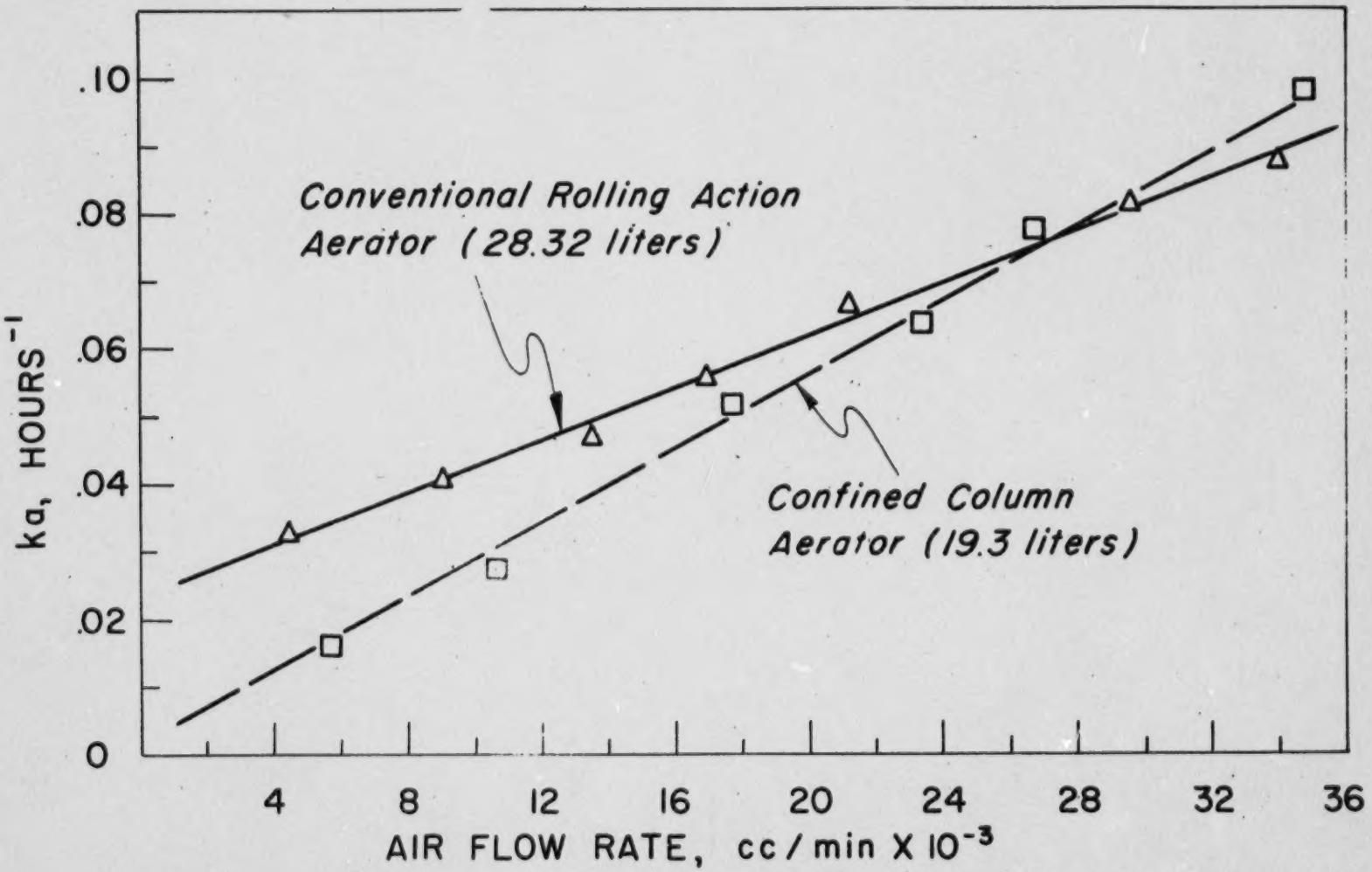


FIGURE 2 - EFFECT OF AERATION TANK GEOMETRY ON THE RELATIONSHIP BETWEEN AIR FLOW RATE AND ka

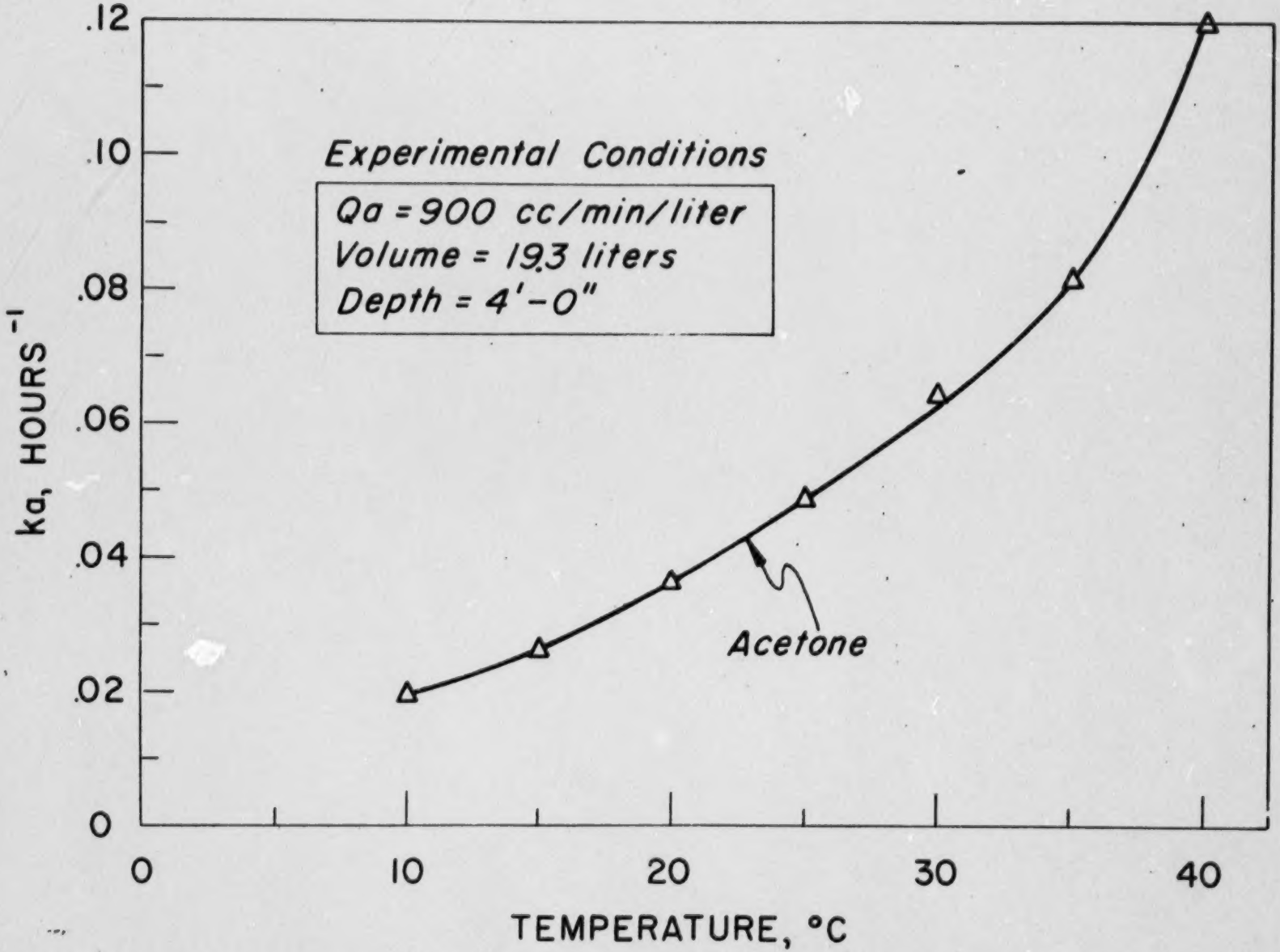


FIGURE 3 - EFFECT OF TEMPERATURE ON ka

express the total removal rate:

$$C_t/C_o = e^{-(ka + kb,)t} \quad (5)$$

where kb, is the first order biological removal rate. On the other hand, if the stripping is first-order and the biological removal is zero-order, Equation 6 expresses the total removal rate:

$$C_t/C_o = e^{-ka(t)} + \frac{kb_o}{C_o(ka)} \quad (e^{-ka(t)} - 1) \quad (6)$$

This equation is valid for a reasonable time interval.

In the evaluation of the separate kinetic constants, centrifugation proved to be a very effective method of obtaining solids-free liquor for filtrate COD analyses. Vacuum filtration of a sample would have resulted in loss of the volatile material in the filtrate. Results also showed that membrane solids determinations made directly on the mixed liquor gave solids concentrations identical with those obtained by centrifugation. A study was made to determine the effect of suspended solids on the over-all stripping transfer coefficient. These studies showed there was no effect for the substrates used.

The Warburg apparatus served as an effective means of studying the biological rate constant in the absence of any physical stripping effects. It was found that stripping in the Warburg apparatus at the substrate concentrations and temperatures employed in these studies was negligible and that COD removal would truly reflect that due solely to biological metabolism.

An acclimated sludge was developed for each of the substrates. The physical stripping and biological removal constants were evaluated separately and then a study was made in which their combined effect was noted. This provided a check between the predicted and actual results. Figure 4 shows the

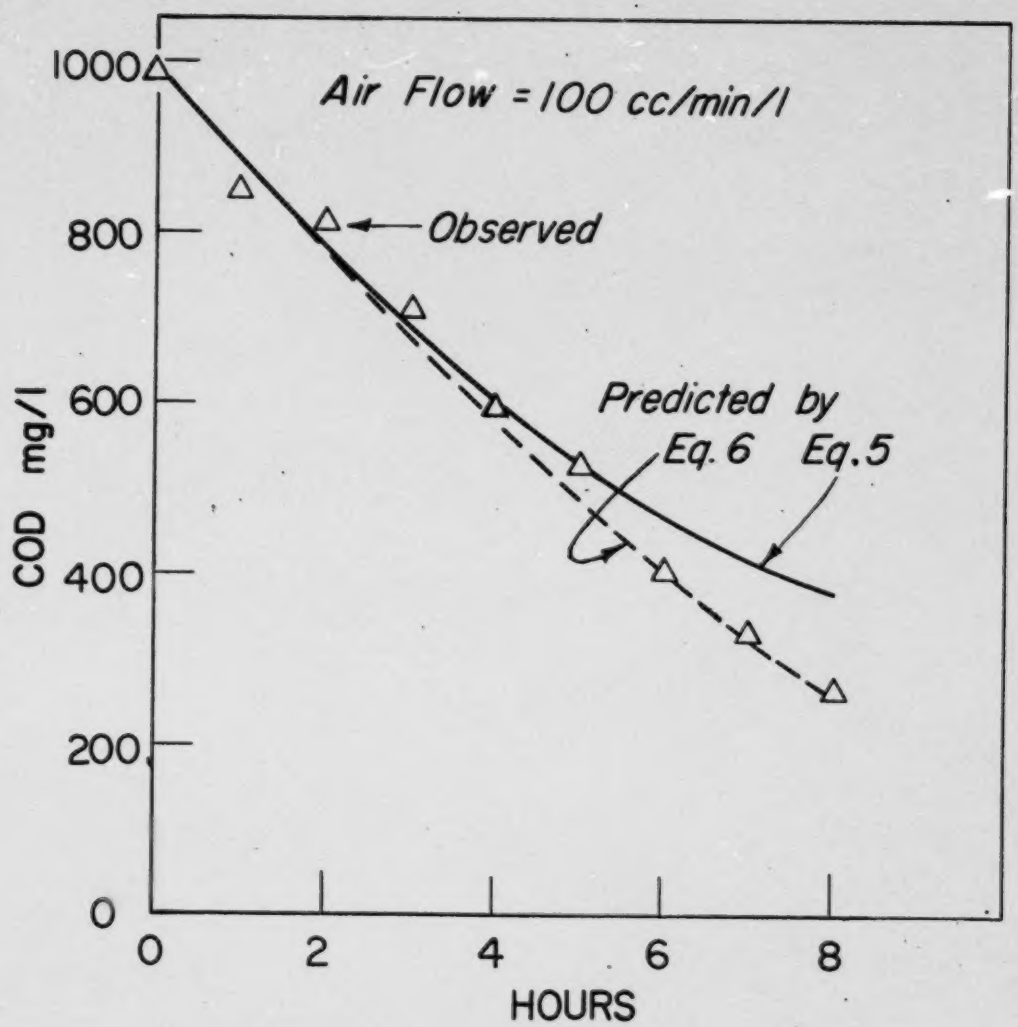


FIGURE 4- PREDICTED & OBSERVED REMOVAL OF ACETONE IN ACTIVATED SLUDGE AERATION TANK

good correlation which resulted between the actual results and those predicted.

Although there are valid technical considerations which militate against accurate prediction of substrate removal by the dual biological and stripping mechanisms, the results indicate that the techniques employed were adequate for such prediction with reasonable accuracy over a fairly wide range of substrate concentrations.

The range of air flow rates in these laboratory studies was from 100 to 1200 cc/min/ℓ (13 to 160 ft^3/min/1000 gal.). This greatly exceeded the practical rate of application of air in a conventional activated sludge plant, which is about 16 cc/min/ℓ (2.28 ft^3/min/1000 gal.). If one were using diffused aeration for physical stripping of volatile compounds, either solely or in conjunction with biological treatment, one might use higher rates of air flows. It is not considered likely, however, that economical use of air could be obtained at more than three times the conventional rate, or 48 cc/min/ℓ (6.5 ft^3/min/1000 gal.). Even such a large increase in air flow rates would not materially increase the rate of removal of the compounds studied (acetone and butanone) in a conventional aeration tank because most of the transfer occurs at the surface of the tank due to mixing provided by the diffused aeration and is not transferred from the bubbles as they ascend. Within practical limits about 30 percent of these compounds might be expected to be removed by air stripping in 6 hours of aeration in a conventional aeration tank. The combined processes of stripping and biological treatment would have removed 50 percent of the acetone and 75 percent of the butanone in the conventional 6 hours of aeration with the solids concentrations used. Figures 5 and 6 depict the expected removals of acetone and butanone, respectively, with respect to aeration time.

From these studies it is obvious that nothing practical is gained by increasing air flow rate beyond the conventional 700 cubic feet per pound

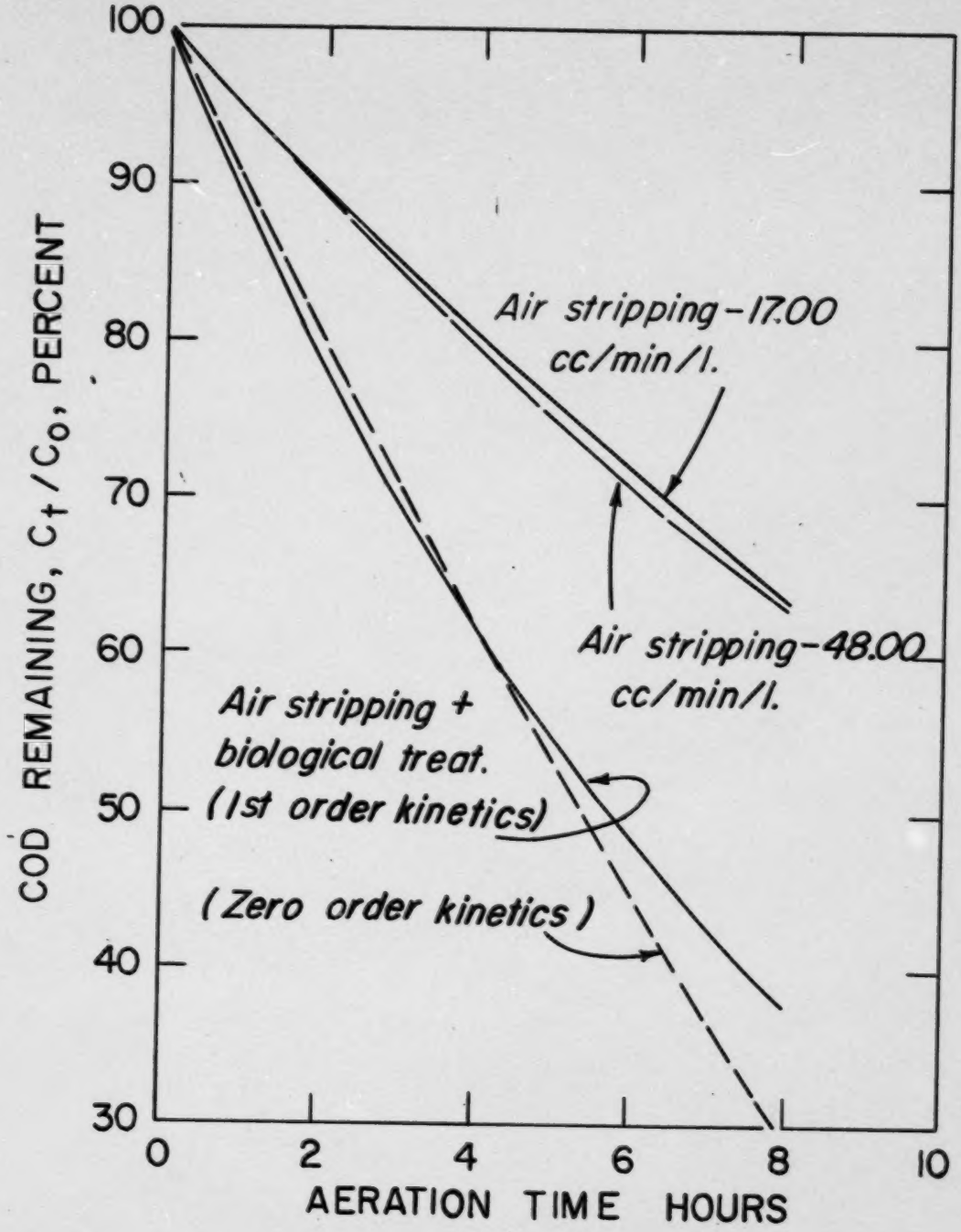


FIGURE 5 - REMOVAL OF ACETONE

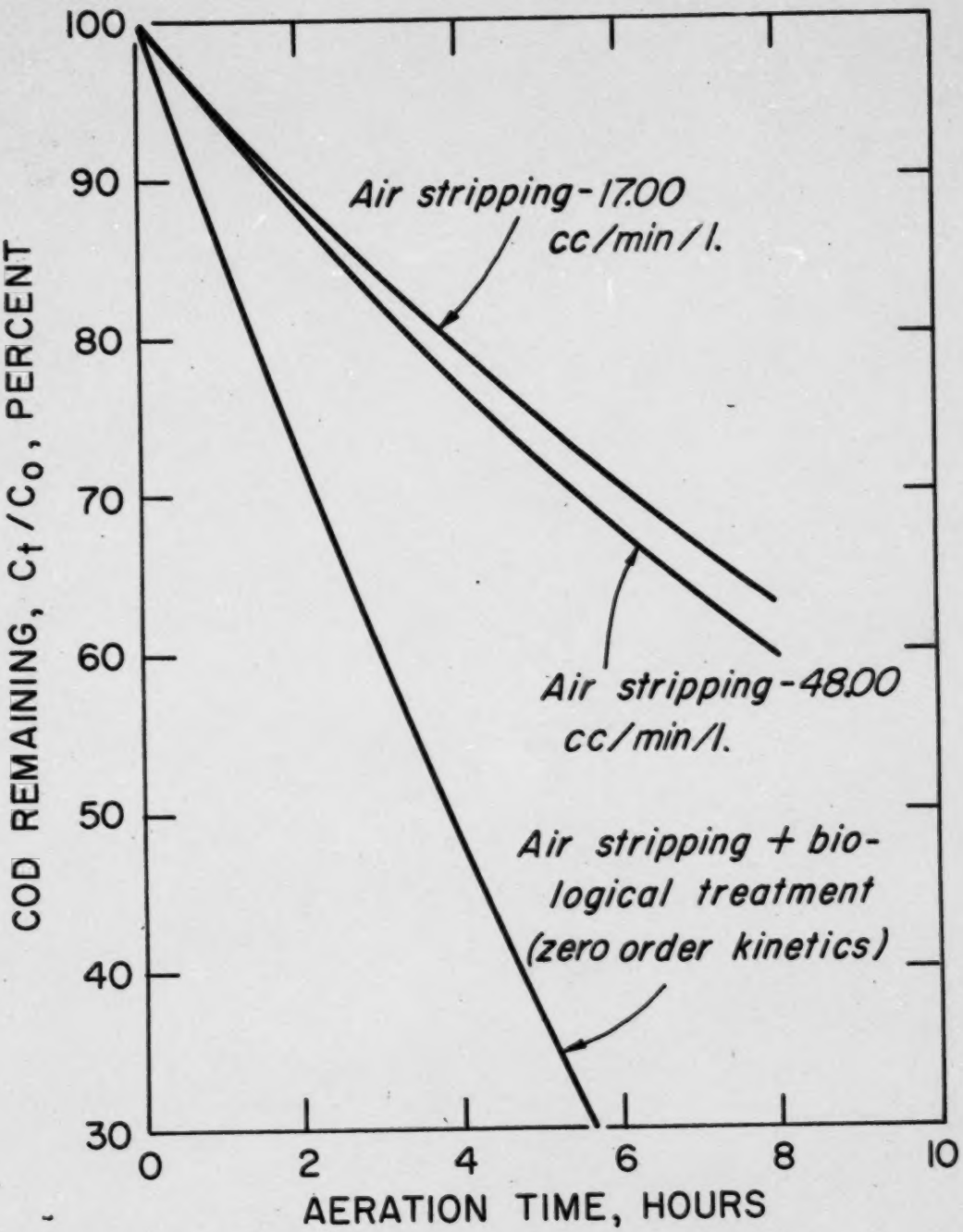


FIGURE 6 - REMOVAL OF BUTANONE

of BOD removed. If sufficient air is applied to obtain good "rolling action" turbulence, then as much as three to four times this amount of air would not significantly improve the removal of volatile compounds. Also, few constituents in a composite petrochemical waste are likely to be as volatile as the substrates acetone and butanone which were used in this study of combined physical stripping and biological treatment phenomena.

Finally, it is recommended that, if the activated sludge process is to be used for biological treatment of petrochemical wastes, the plant should be designed for biological removal only and no allowance should be made for physical stripping unless laboratory studies indicate significant stripping can be effected. Any small amount of stripping which occurs would simply provide a small factor of safety against overloading.

PART 2

TREATMENT OF NITROGEN DEFICIENT WASTES BY A MODIFICATION OF THE ACTIVATED SLUDGE PROCESS

ABSTRACT

A method of treating nitrogen deficient wastes with a modification of activated sludge is proposed. Active bacterial cells are grown in one unit with nitrogen supplementation, and the excess sludge from this unit is utilized in the activated aerator to remove C.O.D. without any nitrogen supplementation.

An F/M ratio of 4 was found to be optimum for the nitrogen supplemented unit whereas a ratio of 2 is needed for the activated aerator. This would save 50 percent of the nitrogen cost. High synthesis is encouraged which results in savings of oxygen consumption compared to a conventional activated sludge. The time taken for 90 percent C.O.D. removal was 8 hours or less in many cases.

Equations were presented for predicting the excess sludge with glucose, phenol and acetate substrates under nitrogen supplemented and nitrogen deficient conditions.

I. INTRODUCTION

A. Nature and Importance of the Problem

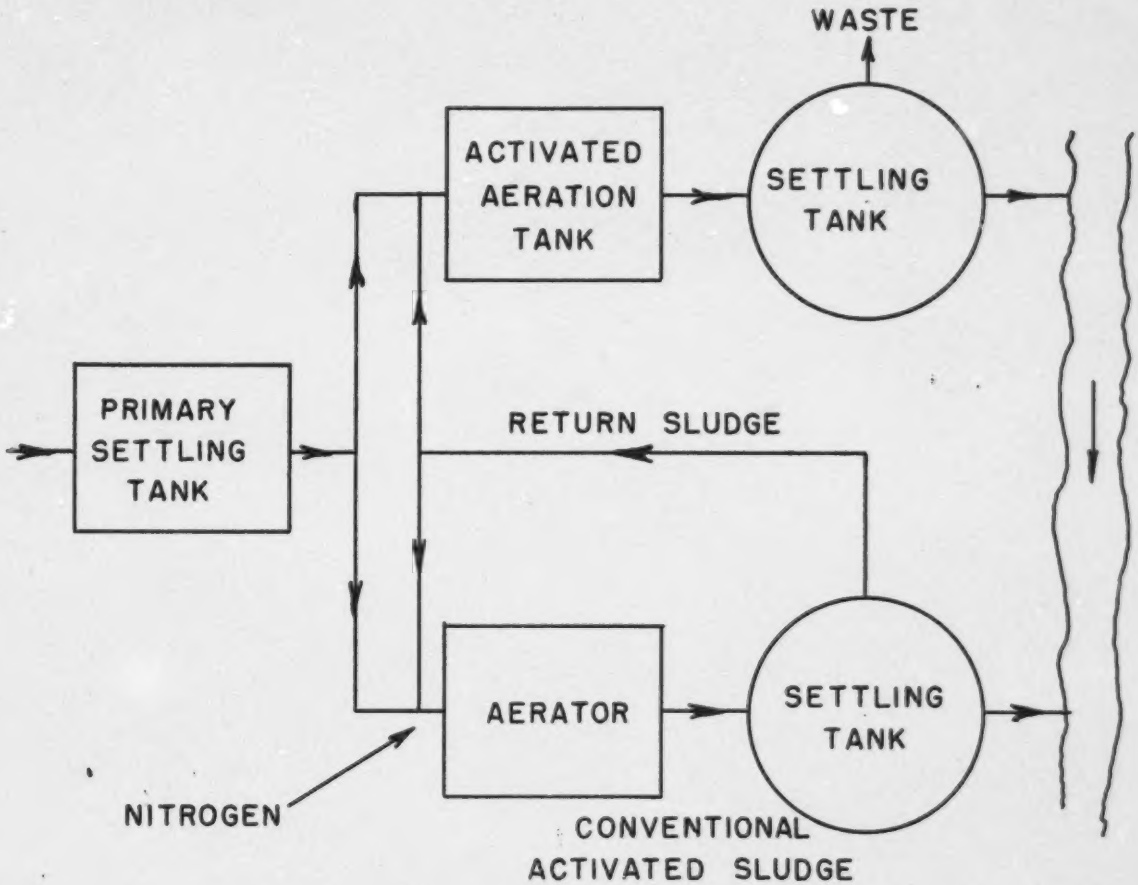
During the last two decades, industrial wastes have contributed increasingly to waste disposal problems. In many places industrial wastes are treated in conjunction with the domestic sewage. On the other hand, many sanitary districts have restricted or refused to accept the industrial wastes because of the treatment problems encountered. A single industry frequently discharges liquid waste with an organic load exceeding that contributed by a whole city of 25,000 to 50,000 inhabitants.

Many of the chemical industries have considerable volumes of liquid wastes. Since the chemical reactions are not usually 100 percent efficient, a part of the various raw materials, reactants and products finds its way into the waste waters. Drinking water resources are becoming heavily polluted and it is becoming more imperative that these wastes be treated to reduce the pollutional load on the streams. Otherwise, these effluents pose a threat to the accustomed high quality of water available for consumption and necessary for continued industrial expansion.

The petrochemical industries along with many others discharge a variety of organic materials. A common peculiarity of these wastes is that they are high in B.O.D. value and deficient in certain essential elements needed for normal biological stabilization. Nitrogen is the principal element often found to be deficient for the biological stabilization of industrial wastes. The sanitary engineer is confronted with such problems and is entrusted with the task of economically treating such wastes.

B. Purpose and Scope of the Study

The object of this work was to determine the feasibility of treating petrochemical wastes, deficient in nitrogen, by a modification of the activated sludge process shown schematically below.



It is known that bacterial cells can be placed in a nitrogen deficient substrate and aerated with subsequent reduction in filtrate chemical oxygen demand (C.O.D.) and increase in mixed liquor suspended solids (M.L.S.S.). It is reported that the substrate is converted into polysaccharides and such a conversion requires less oxygen. The resulting sludge is reported to be resistant to further bacterial degradation. Because of this, the inert polysaccharide sludge could be disposed of easily. Considerable savings

in the amounts of nitrogen to be supplemented are expected in this process.

The process as shown above consists of dividing the primary effluent into two parts. One part is supplemented with a nitrogen source and is treated in a conventional activated sludge process. The excess sludge from this plant becomes the return sludge for the activated aerator. This unit has no nitrogen supplementation. The sludge from this unit after secondary sedimentation is wasted for final disposal.

Petrochemical industries discharge a variety of organic chemicals. A study with a mixture of compounds would not be convenient from the standpoint of process parameters like filtrate C.O.D., M.L.S.S., oxygen uptake, etc. Hence, it was decided to study the process using pure compounds representative of those likely to occur in such wastes. Accordingly, two pure compounds were selected, phenol and acetate. Phenol was chosen to represent the aromatic ring structures and acetic acid was chosen to represent the aliphatic straight chain compounds. A third substrate, glucose, was used as a check to compare with the results of past workers investigating the conventional activated sludge process. An acclimated culture developed on each of the above three substrates was maintained at equilibrium conditions to provide the seed for Warburg studies, which were intended to verify the feasibility of the process.

The scope of the work consisted of determining the oxygen uptake, reduction in filtrate C.O.D. and increase in M.L.S.S. in a nitrogen deficient system, and comparing it with that of a nitrogen supplemented system. An attempt was made to find out the optimum C.O.D./M.L.S.S. (F/M) ratio for maximum process economy with each of the three substrates.

Another phase of the investigation was to find out the duration that a given initial M.L.S.S. concentration would continue to degrade the substrate in the absence of any external nitrogen source and with 100 percent return of the sludge.

II. LITERATURE SURVEY

A. Activated Sludge

The activated sludge process has been one of the best methods of treating wastes for the last 50 years. Fundamentally it is a biological process, in which the heterogeneous spectra of organisms are brought into intimate contact with the waste in the presence of sufficient dissolved oxygen. It has been shown that the organisms utilize the waste for two basic purposes. A portion of the substrate removed by activated sludge is used for cell synthesis and storage products and consumes little oxygen (22). The remainder undergoes oxidation, which is called respiration, to supply the required energy for the metabolism.

$$\text{Total B.O.D. removal} = \underset{\text{(respiration)}}{\text{oxidation}} + \underset{\text{(growth)}}{\text{synthesis}} \quad \ldots\ldots(1)$$

It is known that the respective amounts channelled into the above processes depend on a number of conditions:

(1) The type of organism and its state of activity (phase of growth)

(2) The total number of organisms (initial M.L.S.S.) and its acclimation to the waste

(3) The nutritional aspects of the waste and the availability of dissolved oxygen.

The mechanism of removal of dissolved and suspended solids from the waste has been described in the literature. The major steps in the biochemistry of the oxidative system are summarized by Weston and Eckenfelder (20) as follows:

(1) Initial high rate removal of B.O.D. on contact with biologically active sludge due to reactions between the enzymes and the organic constituents.

(2) Removal of B.O.D. in direct proportion to cell growth.

(3) Oxidation of biological cell material (endogenous respiration) with concurrent low rate of B.O.D. removal.

Activated Aeration: Chasick (1) has described a modification of the activated sludge process called activated aeration. The process was an outgrowth of the idea of re-utilizing the excess activated solids wasted from the secondary settling tank of a conventional activated sludge process. The object of this modification was to partially treat more sewage and obtain greater B.O.D. removal with less total oxygen uptake. The activated aeration system, shown diagrammatically earlier, was used in New York to treat domestic sewage having low B.O.D. The effluent was of intermediate quality and suitable for the local conditions.

A portion of the primary effluent was treated in a conventional activated sludge unit. The excess sludge wasted from the conventional activated sludge process was active and still capable of utilizing organic matter. Activated aeration took advantage of this capacity of the waste sludge to remove organic material on a one-pass, no-recycle basis. The waste sludge was transferred to another aeration tank called an activated aerator into which the remaining portion of the primary effluent was admitted. The M.L.S.S. concentration was very low in the tank (75 mg/ℓ). The contents were aerated for two hours and allowed to settle. There was good synthesis and the final M.L.S.S. were 225 mg/ℓ. The sludge from this process was wasted. The B.O.D. removal was 70 to 75 percent and the overall

efficiency of such a split treatment was about 82 percent. Considerable savings in air compression power costs and reduction in excess sludge volume were claimed to be the advantages of this process. According to Torpey and Chasick (18) the efficiency of the plant was slightly improved when the detention time was increased to four hours. Process stability was said to have been achieved and flexibility of operation realized. This system has worked well with dilute sewage in New York for the last eight years.

B. Nutritional Requirements of Activated Sludge

It is generally conceded that the removal of organic pollutants from waste water is accomplished primarily by bacteria and that rapid removal depends upon unrestricted bacterial reproduction. The removal rate would be greatest when the waste is properly balanced nutritionally. For the bacteria in activated sludge, in addition to a carbon source, nitrogen, phosphorus and sulfur are considered to be the elements needed in measurable quantities. The carriage water generally contains the necessary sulfur in the form of sulfates and also other trace elements. Domestic sewage is nutritionally balanced since it contains excess nitrogen and phosphorus. With industrial wastes, much attention has been given to the nutritional aspects so that they can be successfully treated by the activated sludge process.

Helmers et al. (8), while working with sulfite liquor, reported a B.O.D./nitrogen ratio of 20 and B.O.D./phosphorus ratio of 100, was necessary for successful removal of waste by the activated sludge process. They also reported that the sludge filtering characteristics may influence the amount of nutrients to be added. Poorer floc formation and less B.O.D. removal

was reported by Greenberg and Kaufman (6) in phosphorus deficient wastes. Critical phosphorus requirement for development of activated sludge was given as 1/238 of the B.O.D. Helmers et al. (9) reported that the nitrogen requirement directly affected the B.O.D. removal and sludge growth. Rate of B.O.D. reduction was not affected until the ratio of nitrogen/volatile matter fell below 7 percent. The critical nitrogen requirement for cotton-kiering waste was reported as 3-4 lbs/100 lbs B.O.D. removal.

The nitrogen requirement has been observed by Weinberger (19) to decrease with increasing aeration solids for a given B.O.D. loading due to less synthesis. The nitrogen requirement diminished rapidly as B.O.D. loading decreased and increased to maximum of about 5 lbs per 100 lbs of B.O.D., regardless of the aeration solids concentration. All of the above information was for conditions allowing cell reproduction, and normal protoplasm formation was the result.

Available Nitrogen: According to Sawyer (19) only nitrogen present in the form of ammonia is considered as 100 percent available for the bacteria. Urea must be hydrolysed before it is available as a nitrogen source. Helmers et al. (8) found that nitrogen present in the form of organic nitrogen was not completely available. They indicated 30 to 70 percent of the organically bound nitrogen was available to the bacteria, depending on the character of the waste. Symons and McKinney (17) worked with activated sludge using acetate as the substrate and found that nitrogen in any of the three forms, ammonia, nitrate, and nitrite, could be used as a source of nitrogen.

Symons and McKinney (17) also concluded that even in the absence of nitrogen the purification process continued and the sludge removed the substrate. However, the final product of the bacterial metabolism was no longer true protoplasm but a different product, high in polysaccharides.

Symons (17) has shown that partial satisfaction of the nitrogen requirement could still result in a stable system. Holme (10) has shown that in an E. coli culture, the glycogen content increased from 2 to 20 percent when the cells were subjected to nitrogen starvation. Gaudy and Engelbrecht (4) reviewed the major differences in system behaviour for nitrogen deficient systems (respiring conditions) and nitrogen balanced systems (growth conditions). They concluded that, regardless of the presence of extra-cellular nitrogen, the organisms responded to shock loads, resulting in a rapid increase of sludge mass. It was evident from their studies that for a temporary successful removal of B.O.D. by active cells, no nitrogen source need be supplied. They proposed the possibility of an amino acid pool existence within the bacterial cell. The necessary enzymes were produced to metabolize the waste using this amino acid pool, in the absence of an external nitrogen source. However, the end product of metabolism was high in carbohydrate content and low in protein.

C. Polysaccharide Production and C.O.D. Removal in the Absence of Nitrogen

Wilkinson (21) studied, in pure culture, the influence of growth limiting nutrients other than carbon and energy source on the production of polysaccharides by bacterial cells. He selected type 54, of Klesibella aerogenes because of its simple growth requirements. When he lowered the level of nitrogen source in the medium until it became limiting, the amount of polysaccharides produced per cell rose to a maximum level. This increase was reflected in both the intracellular and extra-cellular polysaccharides. When the experiment was repeated with phosphorus and sulfur source similar results were obtained. In all cases polysaccharide production, as measured by the polysaccharide to nitrogen ratio of the cells, reached a maximum.

The value of this maximum varied according to the growth limiting nutrients. He gave the following values for the maximum.

Polysaccharide : Nitrogen	32:1
Polysaccharide : Phosphorus	40:1
Polysaccharide : Sulfur	17:1

Symons and McKinney (17) have shown that acetate can be metabolized even in the absence of a nitrogen source. The C.O.D. removal was good and the solids accumulation was more than in conventional activated sludge units. Such a system worked for 3 to 4 weeks, before it failed, with 100 percent return of the sludge. Earlier Helmers et al. (9) mentioned that wastes were stabilized even with a limiting nitrogen source but the resulting sludge was not good for vacuum filtration. Gaudy and Engelbrecht (3) found with glucose sludge, that the increase in sludge concentration under respiring conditions (in the absence of nitrogen source) was nearly parallel to that found under growth conditions. More of the substrate removed was accounted for by synthesis under respiring conditions. Under growth conditions, the increase in sludge mass was primarily attributed to creation of new cells; whereas in the case of the nitrogen deficient systems, polysaccharides accumulated in the sludge. Moreover, in both systems the organic load was removed at essentially the same rate and with the same solids production. There was a net accumulation of polysaccharides in the system deficient in nitrogen and operated without wasting, as shown by Symons and McKinney (17). However, the accumulation of solids depended on the time of aeration, initial M.L.S.S. and type of substrate.

Gellman and Heukelekian (5), after studying the relationship between B.O.D. loading and volatile suspended solids accumulation for several industrial wastes, formulated the relationship

$$S = a L_r - b S_a \quad \ldots\ldots\ldots\

S = Resultant net accumulation of VSS (lbs)

L_r = B.O.D. removed in lbs/day

S_a = M.L.V.S.S. in lbs

The constant, a, represents the fraction of B.O.D. removed which is synthesized into new biological sludge. The constant, b, is the coefficient representing the mean rate of endogenous respiration expressed as a percent of the total M.L.S.S. per day.

The results of Symons and McKinney (17) would indicate that the value of, a, will increase in case of nitrogen deficiency. Wuhrman (22) concluded that excess sludge produced by sludge growth is necessarily a function of the same factors which govern optimum purification performance. At a constant plant loading it is expected that excess sludge will increase with decreasing mixed liquor concentrations until the sludge load exceeds the metabolic capacity of organisms. With the same B.O.D. loading net sludge production at M.L.S.S. of 800 mg/ℓ was 67 percent higher than at 3200 mg/ℓ of solids at a temperature of 20° C.

D. Air Requirement of Activated Sludge

The activated sludge process is an aerobic process requiring an oxygen residual of at least 0.5 mg/ℓ according to Porges et al. (13) if the system is to operate properly. The demand for oxygen is a direct function of biological metabolism and is characteristic of the sludge mass and waste. The total oxygen requirement of a bio-oxidation system was given by Eckenfelder and O'Connor (2).

Oxygen requirement/day = a' (B.O.D. removed/day) + b' (M.L.V.S.S.) .....(3)

a' = fraction of the B.O.D. removed, which is used to provide the energy for growth and is equal to (1 - a)

b' = coefficient representing the endogenous respiration rate.

For a variety of industrial wastes the value of a' had been found to be 0.35 - 0.55.

Factors Affecting Oxygen Uptake: Wuhrman (22) while working with glucose acclimated sludge, brought the bacterial cells, which were previously washed in phosphate buffer, so as to be free from any external nitrogen, in contact with the same substrate in the absence of a nitrogen source. He found 15 to 18 percent of the substrate has been respired. The rest was synthesized into new sludge. This was oxidative assimilation in the absence of an external nitrogen source. However, the percentage respired increased to 50 percent in the presence of ammonia nitrogen. He concluded, this was due to the assimilation of ammonia which is a process of high energy expenditure. He also concluded that the amount of oxygen consumed by sludge during removal of a certain fraction of oxidizable nutrient (sugar) from solution, may vary within wide limits depending on the other metabolites present such as nitrogen. Such wastes, when supplemented with an assimilable nitrogen source, might exert very high oxygen demand. Thus the availability of nutrients will influence the coefficient, a', in equation 3. This was confirmed in the work of Symons and McKinney (17) in which oxidation in the presence of low nitrogen resulted in a large accumulation of sludge of high polysaccharide content and, hence, in a lower fraction oxidized.

The concentration of trace elements often exhibits a far more pronounced effect on oxygen uptake. Mg^{++}, Mn^{++}, Fe^{++}, etc., form co-factors for respiratory processes. Their absence will result in lower oxygen consumption according to Oginsky and Umbreit (12). For the same B.O.D. load, oxygen uptake varied with the initial M.L.S.S. The growth phase of bacteria also influences the oxygen uptake.

Oxygen Uptake Rate: The oxygen uptake rate will vary with time of aeration as the sludge passes through the various phases, depending on the concentration of sludge employed and the C.O.D. of the waste. If the (F:M) C.O.D. to M.L.S.S. ratio is low, the initial rate will be high. Due to rapid initial removal of C.O.D. under these circumstances, the uptake rate will decrease rapidly and approach endogenous level. If the C.O.D. to sludge ratio is high, a low rate will be maintained for a long period of time as the C.O.D. removal and oxidation process continues. If the sludge is not acclimated to the waste, an initial lag period of low oxygen uptake is exhibited. Later, the waste is metabolized and the oxygen uptake rate increases, once acclimation has taken place.

When the organisms, acclimated to the waste, were thoroughly mixed with the waste, especially with a high M.L.S.S., it was observed that there was considerable reduction in the C.O.D. of the substrate within a short time of contact. This high initial drop in C.O.D. is attributed to adsorption and absorption by Smith and Ullrich (15). Gaudy and Engelbrecht (4) had also concluded that this phenomenon is absorption in the case of soluble substrates. Such a process needs much less oxygen than when the substrate is oxidized.

Storage and Oxidation: Gellman and Heukelekian (5) found the rates of purification (C.O.D. removal) of various industrial wastes to be much higher and even several fold that of their respective oxygen uptake rates. Porges et al. (13) obtained a B.O.D. reduction rate of six times the oxidation rate while working with 1000 mg/ℓ of M.L.S.S. and skimmed milk waste of 1000 mg/ℓ concentration. They found 80 percent of the C.O.D. was removed in one hour while the oxygen uptake corresponded to only 50 percent of the C.O.D. removal, thereby inferring cell storage or absorption. This stored material was utilized subsequently. A portion of the C.O.D.

removed was oxidized to carbon dioxide and water. Another portion was synthesized into cell complex with a low immediate oxygen demand while the remainder is converted and stored as an insoluble glycogen-like substance. However, the oxygen demand of the mixed sludge continues at a high rate while the storage product is utilized and then decreases to the endogenous rate, if aerated long enough.

E. Sludge Characteristics of Nitrogen Deficient Wastes

One of the most important aspects of nutritionally deficient wastes is their effect on biological predomination. A partly nitrogen deficient waste will permit the predominance of fungi over bacteria according to McKinney (11), since fungi form protoplasm with a lower nitrogen content than bacteria. The fungi are filamentous and hinder the settling of sludge. According to Symons and McKinney (17), the polysaccharide sludge was resistant to further biological degradation and was described as inert volatile matter. Helmers et al. (9) have shown that low nitrogen sludges tend to have poor settling characteristics and might impair vacuum filtration.

F. Energy Considerations : Nutrition Limitation

During bacterial respiration, the substrate is oxidized with the release of energy. In an actively metabolizing system this energy is usefully employed by the cell for the synthesis of new protoplasm. When a nitrogen source is absent, the cell is faced with the problem of dissipating this energy, since it is no longer producing new protoplasm. Gunsalus and Stanier (7) described the following three possible mechanisms of energy

dissipation by the cell other than the formation of new protoplasm. (1) Polymeric products like polysaccharides are accumulated either in storage form or as unusable waste. The excess energy is impregnated in the form of high energy bonds into these products. (2) While degrading a substrate, the energy which is given off during the breaking of the molecules, is harnessed by the bacteria in the form of high energy bonds known as Adenosine Tri Phosphate (A.T.P.). This A.T.P. bond is broken by a specific enzyme called A.T.P.-ase, present in the cell. Such a process permits the trapped energy to be dissipated as heat. (3) The cell tries to activate shunt mechanisms bypassing energy-yielding reactions or by requiring a greater expenditure of energy for priming. Priming is defined as the initiation of a biochemical reaction.

Experiments of Senez et al. (14) with nitrogen limited growth showed a depressed growth rate without decreased substrate turnover - e.g. a metabolic rate independent of growth. This would be possible only if some mechanisms either bypassing A.T.P. generation or permitting its dissipation at a uniform rate were present in the cell. This is an example of the third postulate in the previous paragraph.

III. PROCEDURES AND EXPERIMENTAL TECHNIQUES

A. Acclimated Cultures

Activated sludges, acclimated to glucose, phenol, and acetate were maintained in separate tanks. The cultures were maintained in an active state by daily wasting of 2/3 of the total mixed liquor contents of the tank. They were all batch fed daily with 1 gm/ℓ as C.O.D. of the respective substrate. The daily feed is given below:

Substrate as C.O.D.	1000 mg/ℓ
Phosphate Buffer as PO_4, PH.7.0	10 mg/ℓ
NH_4 Cl	500 mg/ℓ
Mg $SO_4 \cdot 7H_2O$	250 mg/ℓ
Fe $SO_4 \cdot 7H_2O$	10 mg/ℓ
Ca $Cl_2 \cdot 2H_2O$	10 mg/ℓ
Mn $SO_4 \cdot H_2O$	10 mg/ℓ

Regular determinations were made for the M.L.S.S. and 23 hour filtrate C.O.D. The settling characteristics of the sludge were observed by shutting off the air flow. Ammonia and organic nitrogen were determined occasionally to check the nitrogen content of the sludge. After equilibrium was established as judged by constant M.L.S.S. and a high fraction of C.O.D. removal, Warburg studies were initiated to verify the efficacy of the process.

M.L.S.S. were determined by the membrane filter technique. However, the C.O.D. was determined by the modified C.O.D. procedure, which uses 1/5 of the recommended quantities, and a titrant of 1/5 of the recommended normality. All other tests were carried out as per Standard Methods (16)

B. Warburg Studies

The procedure for each experiment consisted of withdrawing a volume of the mixed liquor from the seed tank, which had been fed 23 hours earlier and allowing the solids to settle. After the solids had concentrated, the supernatant was syphoned off above the sludge blanket and the solids were washed in a physiological salt solution to remove extra-cellular nitrogen. This solution had the same salt concentrations to which the seed was acclimated except that the nitrogen source was absent. After three successive elutriations, the dilution factor was 250. Hence the ammonia nitrogen content was 1/250 of the original ammonia nitrogen. The approximate concentration of the solids was determined optically using a spectrophotometer. Then the solids were divided into two equal portions. Nitrogen source, in the form of ammonium chloride, was added to one portion at the concentration of 500 mg/ℓ and the concentrated sludge was diluted with the physiological salt solution to the desired concentration of M.L.S.S. The second portion was not supplemented with any nitrogen source and was diluted to the required concentration of M.L.S.S. The M.L.S.S. concentration and filtrate C.O.D. of these seed sludges were determined. The initial filtrate C.O.D. was necessary to determine chloride interference in the seed sludges. The ammonia and organic nitrogen content were determined on each of the seed sludges.

To determine the increase in M.L.S.S., rate of oxygen uptake and reduction of C.O.D. with time under nitrogen deficient conditions, eight Warburg flasks were set up with the same substrate concentration and M.L.S.S. concentration as prepared above. The seed was ten millliliters consisting of all nutrients and a double concentration active cells. Ten millliliters of substrate was added, thus making the flask contents 20 ml in total. The nutrients salts concentration was the same in the flask as that to which the

seed was acclimated. The central well in the Warburg flask was filled with 1 ml of a 10 percent potassium hydroxide solution so as to absorb the carbon dioxide produced during bacterial metabolism. Eight more flasks were set up under the same conditions and supplemented with nitrogen. The manometers of the Warburg apparatus were filled with mercury instead of Brodie solution to permit taking of oxygen uptake readings over a prolonged period of time. The maximum vacuum permitted was 50 mm. The temperature of the water bath was maintained at 25°C throughout the entire study. One flask from each of the above groups was removed at 1 hour or 1.5 hour intervals and analysed for M.L.S.S. and filtrate C.O.D.

One flask was reserved for endogenous respiration from each group. Another flask in each group was kept for determining the ammonia and organic nitrogen at the end of the experiment. The Warburg study was discontinued when the oxygen uptake decreased to the endogenous rate or at the end of eight hours, whichever was earlier.

Three different concentrations for each of the three substrates were selected. They were 500, 1000 and 2000 mg/ℓ as C.O.D. Warburg studies with food : microorganisms (F : M) ratios of 4, 2, 1 and 1/2 were conducted for each of the above concentrations. This was repeated with all the three chosen substrates namely, glucose, phenol and acetate. The procedures followed were identical in all respects.

C. Duration of Substrate Removal with 100 Percent Return Sludge Under Nitrogen Deficient Conditions

Glucose Substrate: In this study four small activated sludge tanks of 1.5 liter capacity were started with thoroughly washed active cells. These cells were taken from the acclimated activated sludge tanks described

earlier. The ammonia-nitrogen content of the M.L.S.S., after three successive elutriations, was approximately 0 mg/ℓ. These four tanks received 100, 200, 600 and 1000 mg/ℓ respectively of washed glucose acclimated sludge. These systems were operated without sludge wasting. The evaporation loss was corrected with distilled water. The pH of the tank contents was adjusted daily to .7. These tanks received at the initiation of the experiment the same amount and concentration of nutrients (described in the previous section) to which they were acclimated earlier with the exception of the nitrogen source. They were all fed daily 1000 mg/ℓ as C.O.D. of the substrate. No more nutrients were added. A sample of 25 ml was taken out daily for the determination of M.L.S.S. and filtrate C.O.D. and this volume was replenished with the physiological salt solution. An extra 25 ml of sample was withdrawn on alternate days for the determination of ammonia and organic nitrogen. This volume was replaced with distilled water.

The above study was repeated with phenol and acetate acclimated cultures and their respective substrates under the same conditions as described above.

This study was terminated when there was no further degradation of the substrate as indicated by the accumulation of substrate for at least three days. The criteria for the accumulation was the 23 hour filtrate C.O.D. value.

IV. RESULTS

1. Warburg Experiments

A.1. Glucose: substrate concentration 500 mg/ℓ as C.O.D. With this substrate concentration, four experiments were conducted with approximate initial M.L.S.S. of 100, 280, 440 and 1070 mg/ℓ.under both nitrogen supplemented and nitrogen deficient conditions. The last two experiments are shown on Figures 1 and 2 depicting the C.O.D. removal, oxygen uptake and increase in M.L.S.S. with respect to time. The other relevant data are shown in Table I.in the Appendix. The results with 100 and 280 mg/ℓ initial M.L.S.S. under nitrogen supplemented conditions are shown subsequently in the section on synthesis of protoplasm. The results with the nitrogen deficient systems under identical conditions, were rejected and not included, since 90 percent C.O.D. removal was not obtained within the prescribed detention time of 8 hours.

A.2. Glucose: substrate concentration 1000 mg/ℓ as C.O.D. Four more Warburg experiments were conducted with approximate initial M.L.S.S. concentration of 250, 500, 1000 and 2000 mg/ℓ under both nitrogen supplemented and nitrogen deficient conditions. All the data are shown in Table I in the Appendix. The results are plotted in Figures 3 - 5. The experiment with 250 mg/ℓ initial M.L.S.S. was not included in the data since it did not satisfy the 8 hour time limit criterion.

A.3. Glucose: substrate concentration 2000 mg/ℓ as C.O.D. The F/M ratios were 4, 2 and 1. The last experiment is included in Table I in the Appendix and shown in Figure 6. The experiments with 500 mg/ℓ, and 800 mg/ℓ of initial M.L.S.S. were not satisfactory so far as 90 percent of C.O.D. removal in 8 hours was concerned and were excluded.

B.1. Phenol: substrate concentration 500 mg/ℓ as C.O.D. Three experiments were conducted with approximate initial M.L.S.S. of 200,

500 and 1000 mg/ℓ. They are shown in Figures 7 to 9. The results are included in Table II in the Appendix.

B.2. Phenol: substrate concentration 1000 mg/ℓ as C.O.D. Four experiments were conducted with F/M ratios of approximately 4, 2, 1 and 1/2 respectively. The study with a F:M ratio of 4 was omitted from the data. However, there were two experiments with a ratio of 2. The results are shown on Figures 10 to 13 and included in Table II in the Appendix.

B.3. Phenol: substrate concentration 2000 mg/ℓ as C.O.D. Only one experiment was satisfactory for inclusion in the data and is represented in Table II in the Appendix and in Figure 14.

C.1. Acetate: substrate concentration 500 mg/ℓ as C.O.D. Four experiments were conducted with approximate F/M ratios of 4, 2, 1 and 1/2. In case of the nitrogen deficient system, the higher ratio did not prove satisfactory and, hence, was not included in Table III in the Appendix. These data are plotted in Figures 15 to 17.

C.2. Acetate: substrate concentration 1000 mg/ℓ as C.O.D. Four experiments were carried out with approximate initial M.L.S.S. of 250, 500, 1000 and 2000 mg/ℓ respectively. The results are given in Table III in the Appendix. Only the last three experiments satisfied the criteria set in this study and are shown in Figures 18 to 20.

C.3. Acetate: substrate concentration 200 mg/ℓ as C.O.D. Only two experiments were conducted with an initial M.L.S.S. of 500 and 1000 mg/ℓ. The system with a F:M ratio of 4, under nitrogen deficient conditions did not satisfy the requirements of 90 percent C.O.D. reduction in 8 hours and, hence, it was rejected. The experiments with F/M ratio of approximately 2 are shown in Figure 21. The relevant data are included in Table III in the Appendix.

2. Operational Characteristics with No Solids Wasting

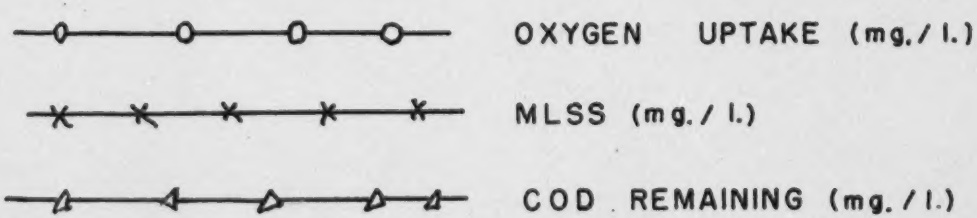
The object of this study was to evaluate the length of time that an active cell mass would continue to degrade the replenished substrate without any nitrogen source. In this study, no wasting of sludge was permitted. The feed was given daily and the 23 hours filtrate C.O.D. and M.L.S.S. were determined daily. The results of this study are given for all the three substrates in Table IV in the Appendix. Ammonia and organic nitrogen determinations were determined on the contents of the tanks on alternate days. The ammonia nitrogen was zero every time for all the units. The C.O.D. removed per day by each of the systems is plotted against time in days and is shown separately for each substrate in Figures 22 to 24.

3. Protoplasm Productions

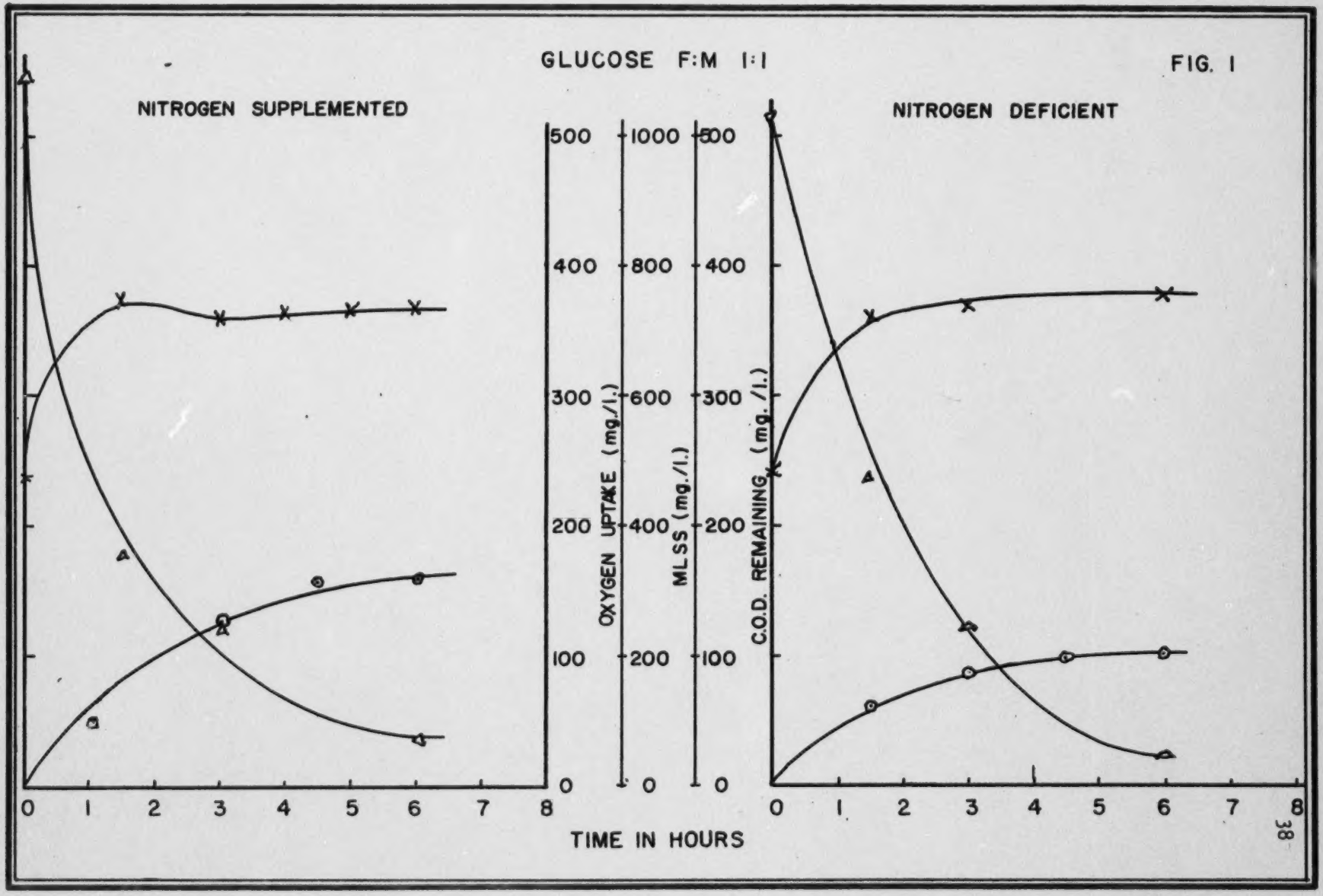
Since the continuous production of active cells is the core of this process, Warburg studies were conducted with low initial M.L.S.S. under nitrogen supplemented conditions to find the favorable F/M ratio for maximum cell production. The substrates were the same as described earlier. The concentrations of the substrates were 500, 1000 and 2000 mg/ℓ. Only the results of those experiments which could satisfy the criterion of 90 percent C.O.D. reduction in 8 hours are shown in Table V in the Appendix. The increase in M.L.S.S., reduction of C.O.D. and oxygen uptake are plotted with respect to time and shown in Figures 25 to 28.

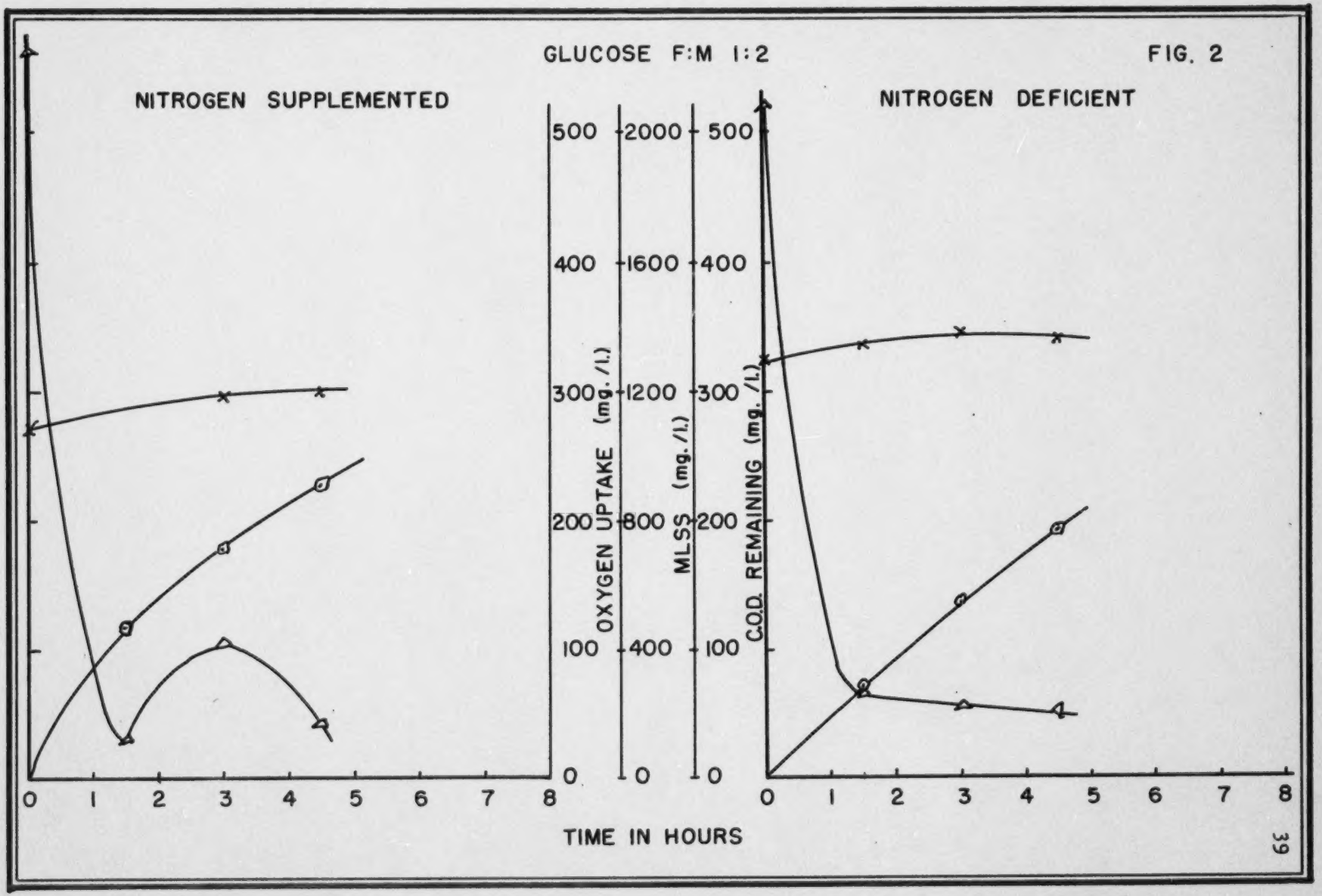
FIG. O

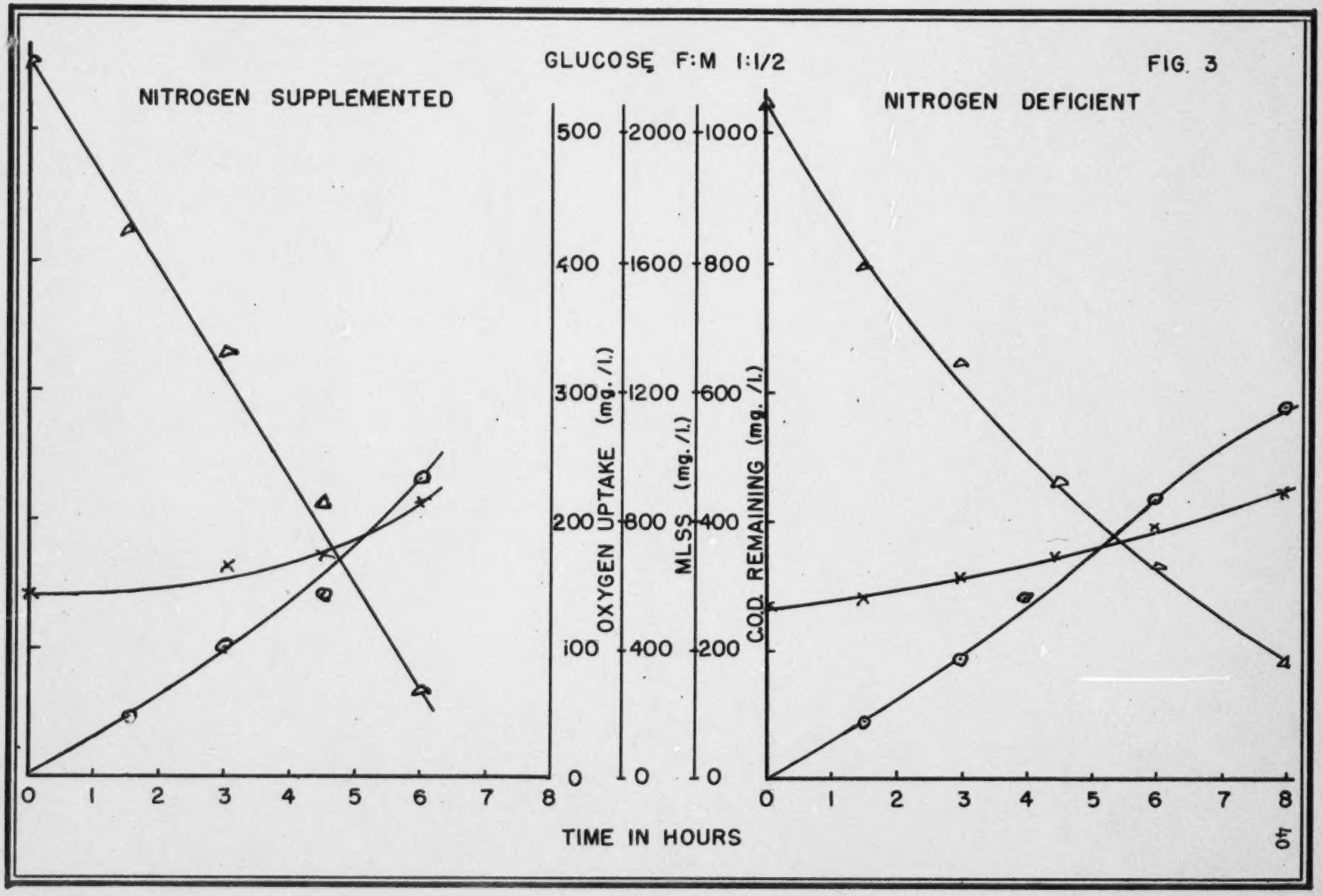
LEGEND

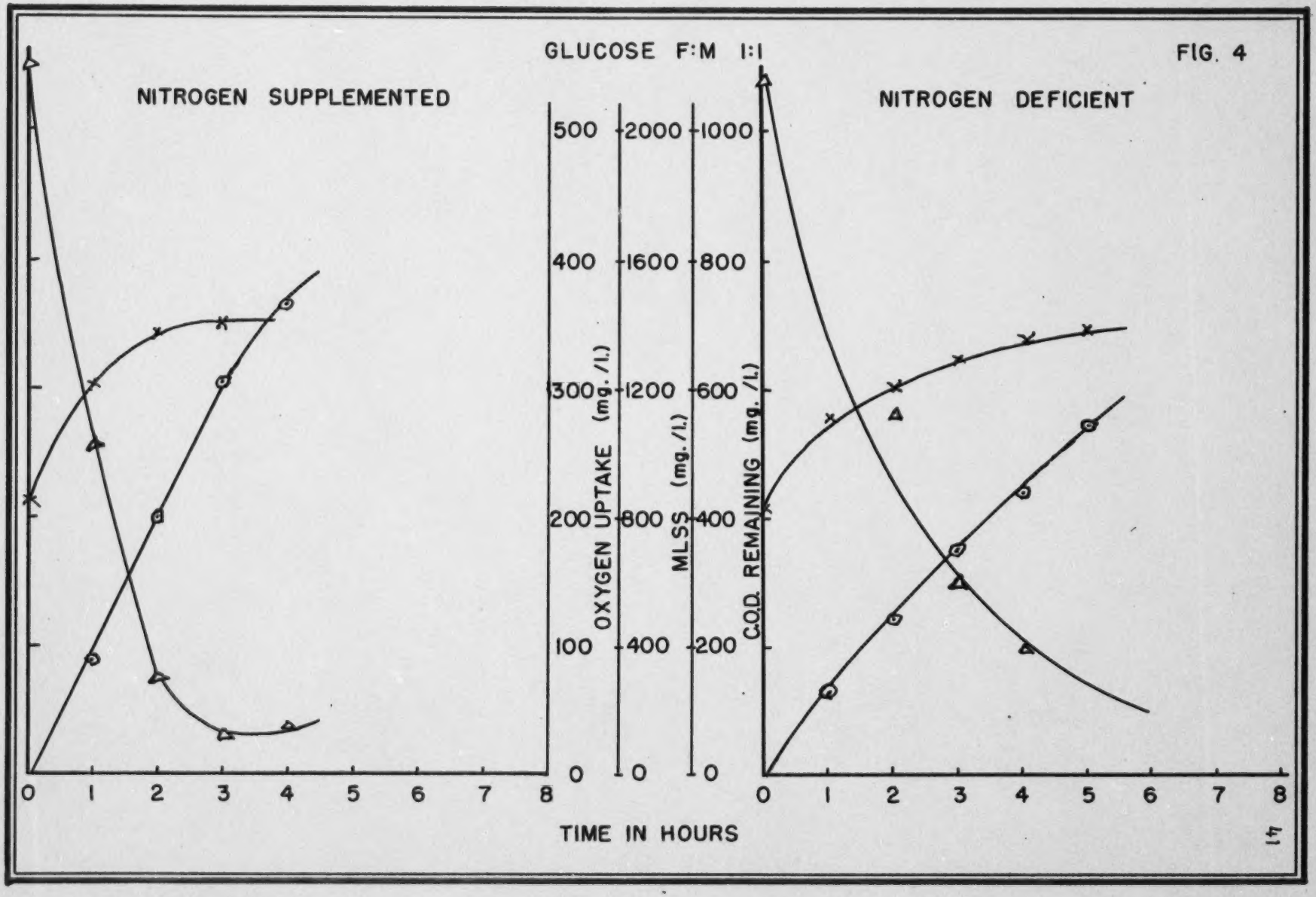


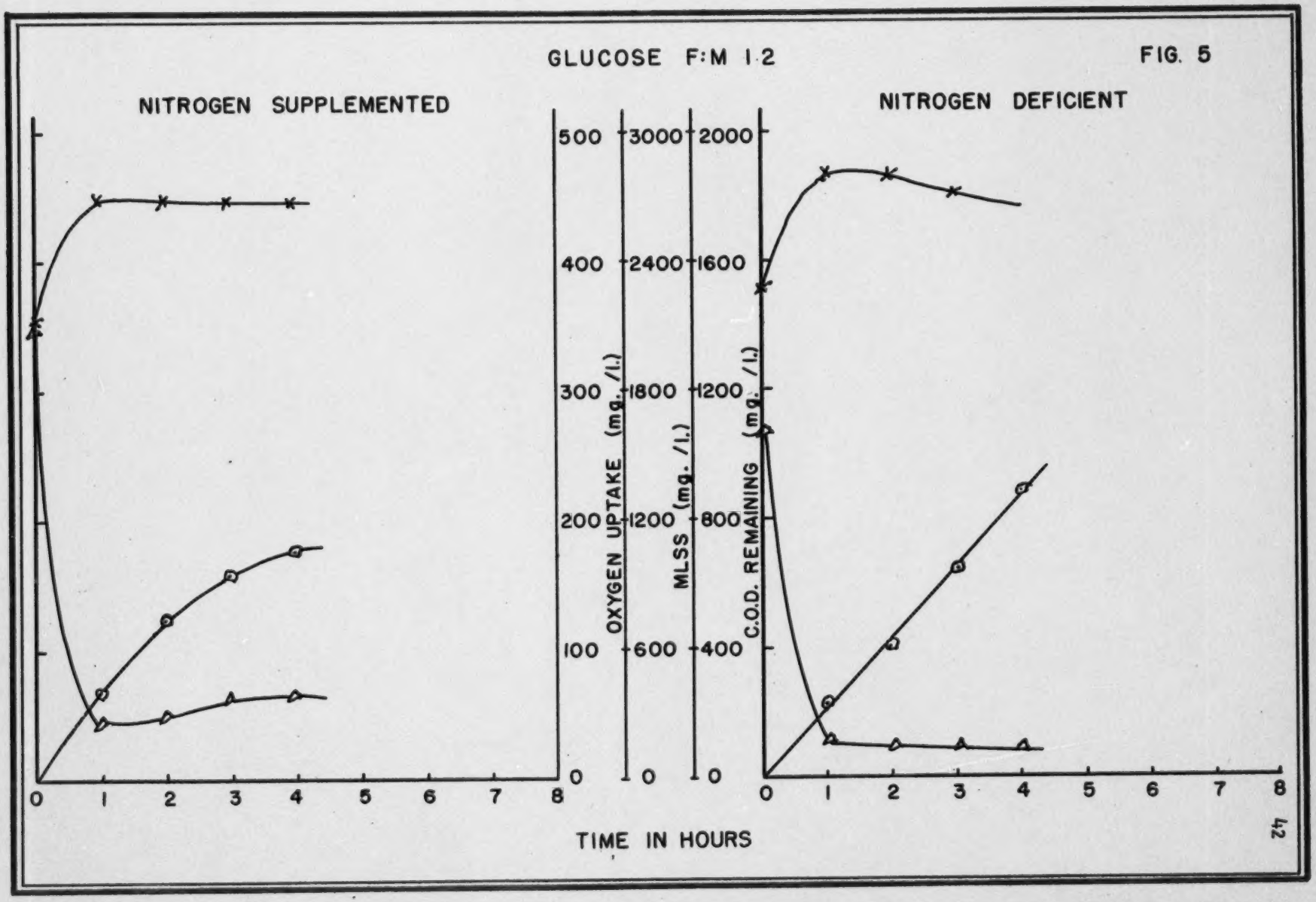
THE ABOVE SYMBOLS HAVE BEEN USED
FOR FIG. I THROUGH FIG 21
AND
FIG 25 THROUGH FIG 28

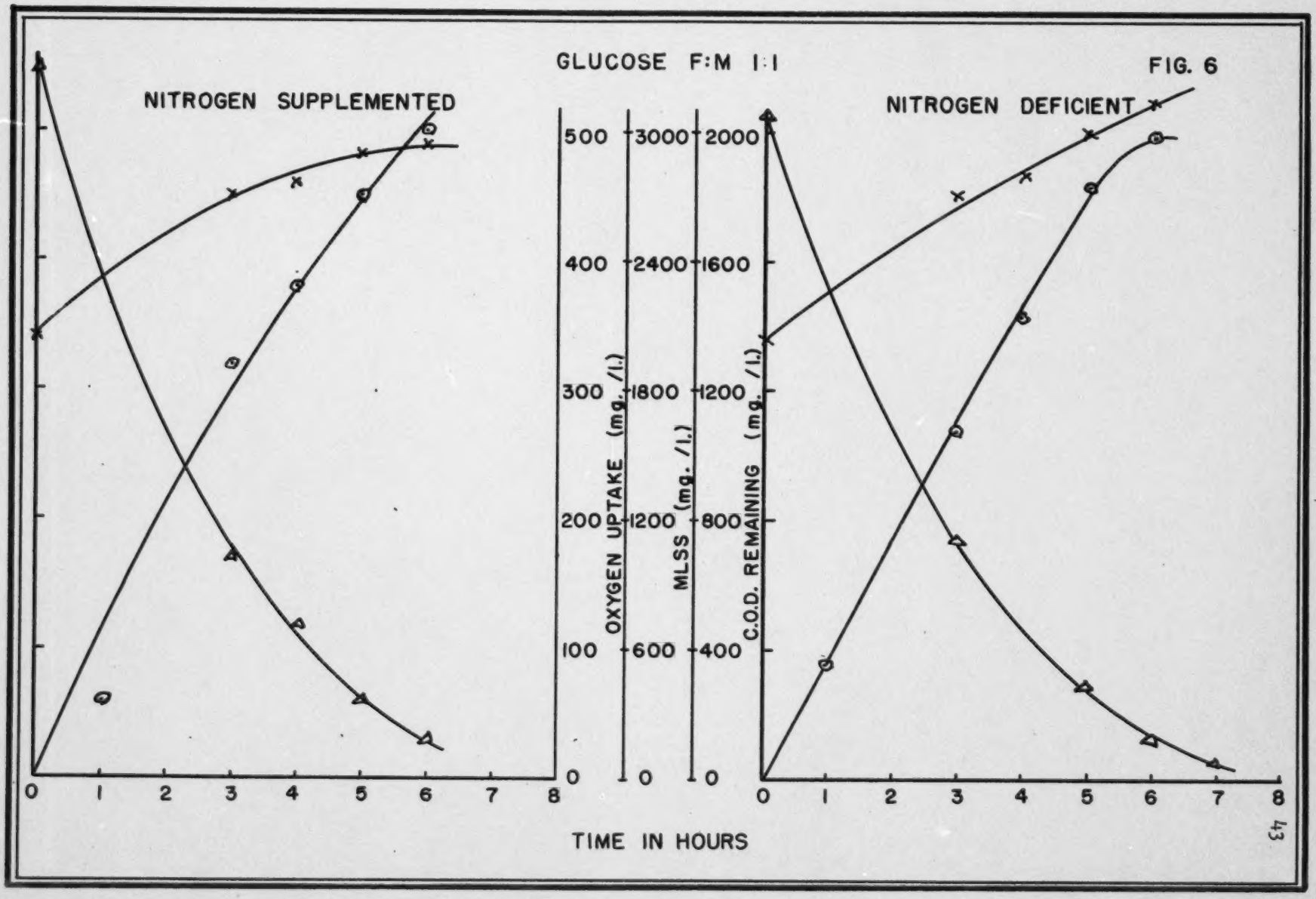

GLUCOSE F:M 1:1
FIG. 1
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./l.)
500
400
300
200
100
0
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS

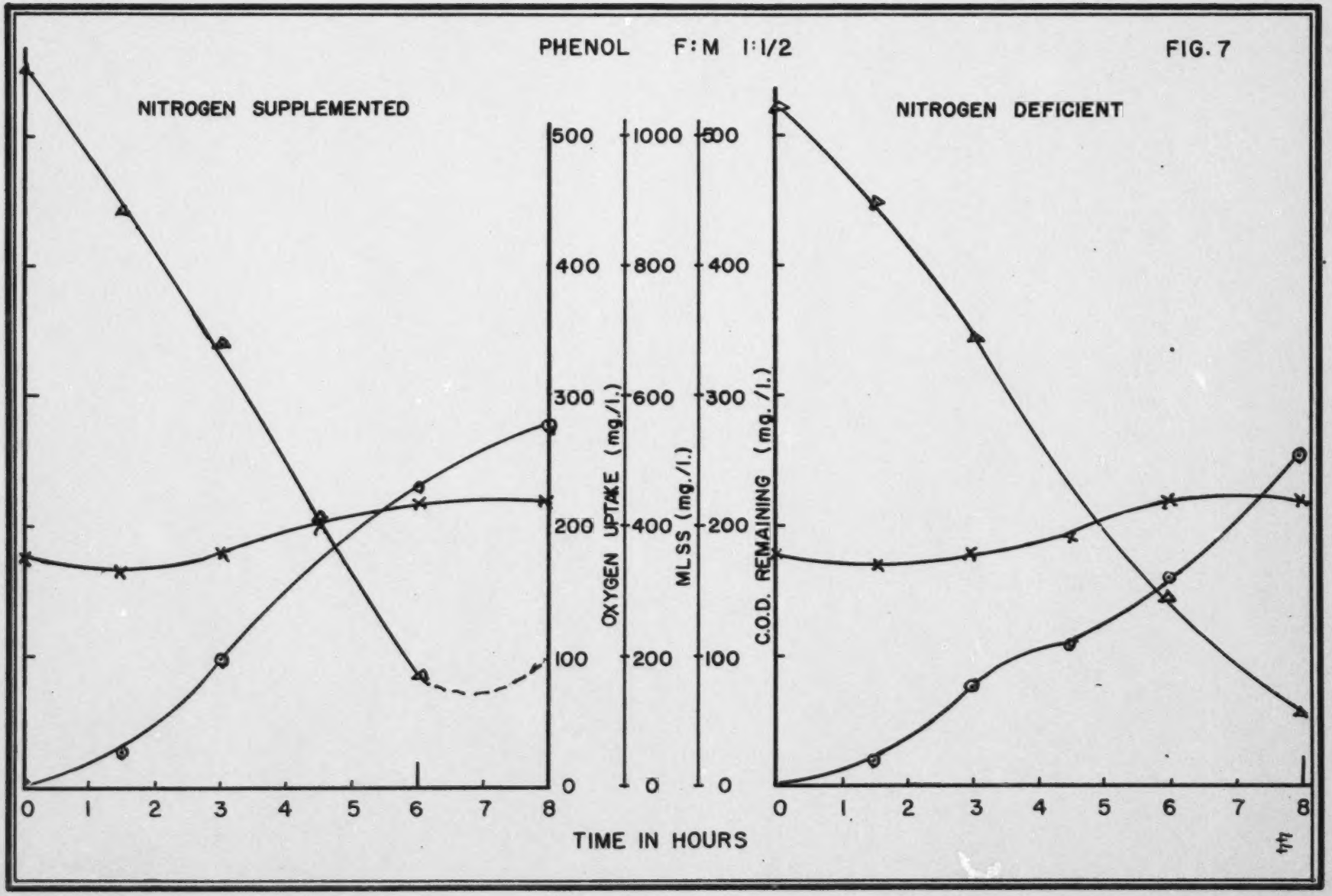

GLUCOSE F:M 1:2
FIG. 2
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./L.)
500
400
300
200
100
0
2000
1600
1200
800
400
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS

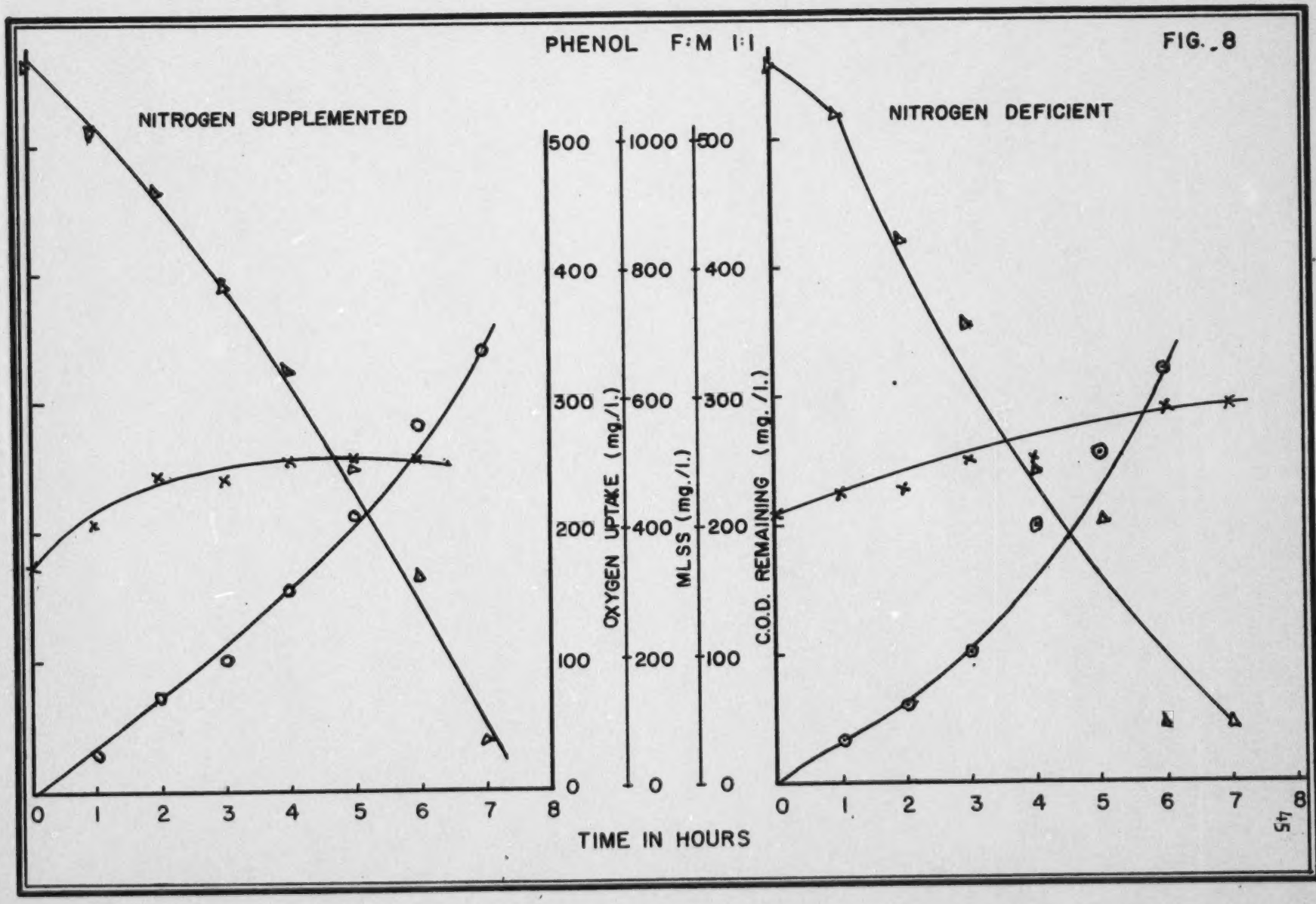

GLUCOSE F:M 1:1/2
FIG. 3
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
COD REMAINING (mg./l.)
500
400
300
200
100
0
2000
1600
1200
800
400
0
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS
40

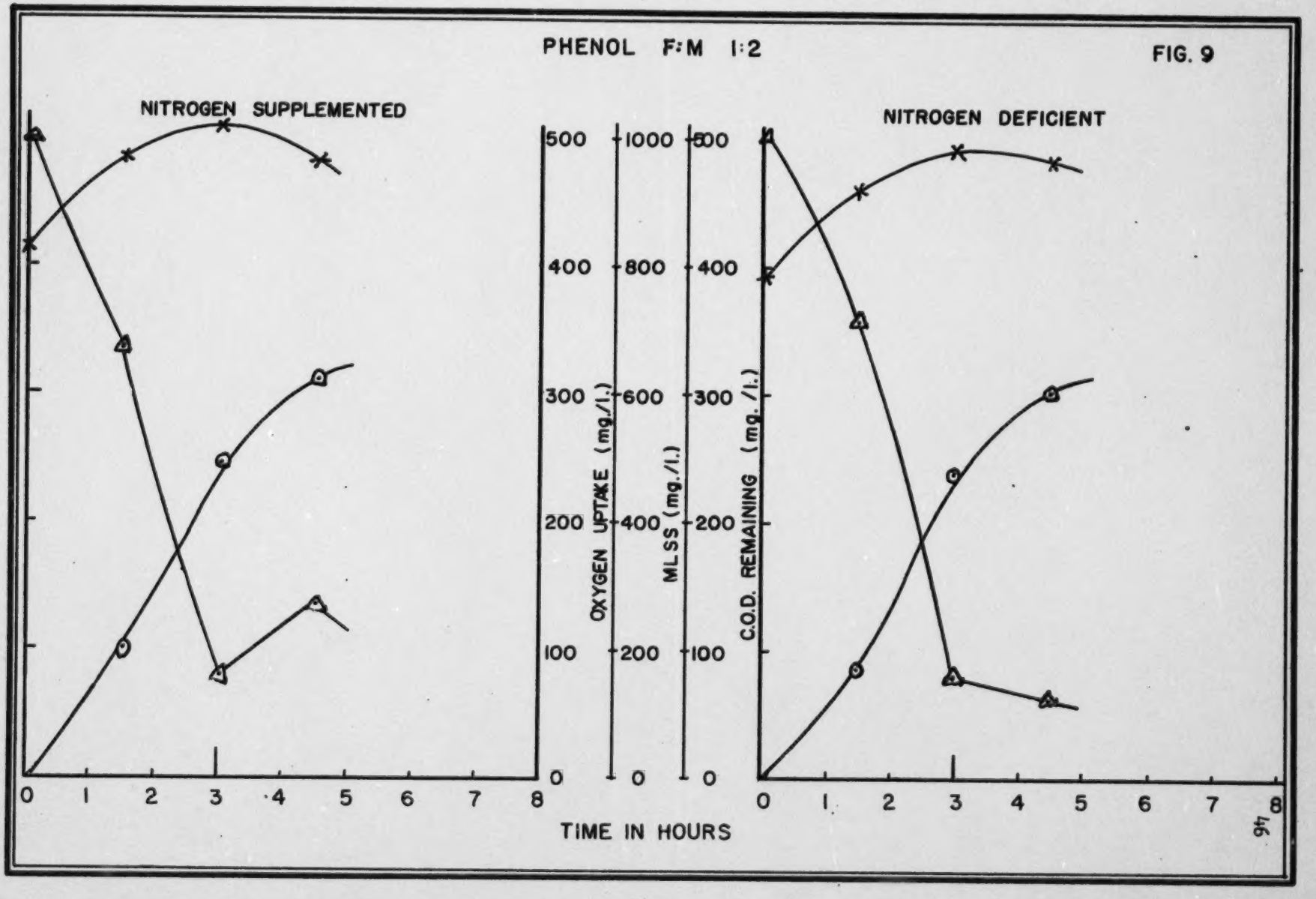

GLUCOSE F:M 1:1
FIG. 4
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
500
400
300
200
100
0
2000
1600
1200
800
400
0
1000
800
600
400
200
0
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./L)
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 6 7 8
TIME IN HOURS
47

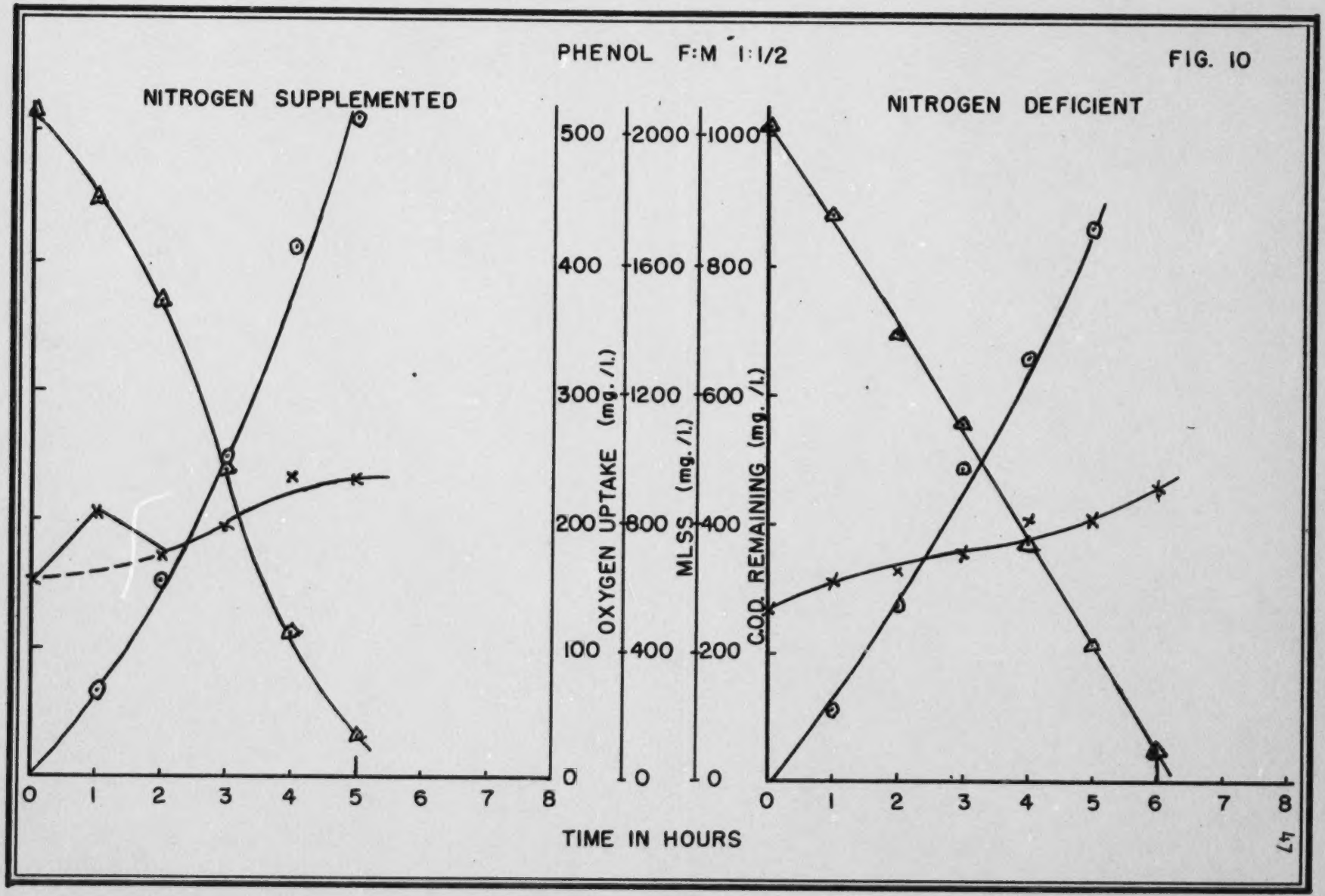

GLUCOSE F:M 1.2
FIG. 5
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./l.)
500
400
300
200
100
0
3000
2400
1800
1200
600
0
2000
1600
1200
800
400
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS
42

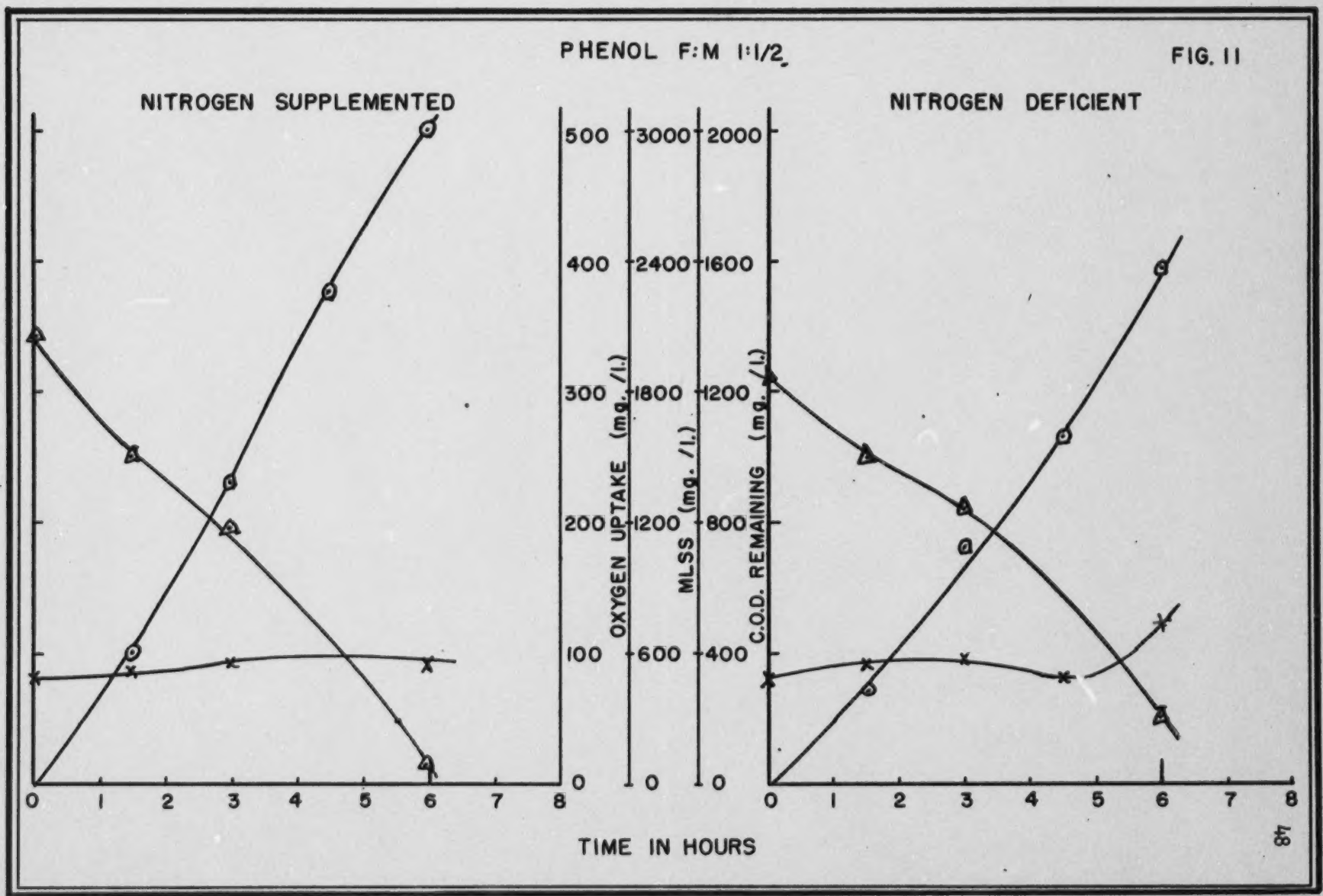

GLUCOSE F:M 1:1
FIG. 6
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./l.)
500
400
300
200
100
0
3000
2400
1800
1200
600
0
2000
1600
1200
800
400
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS

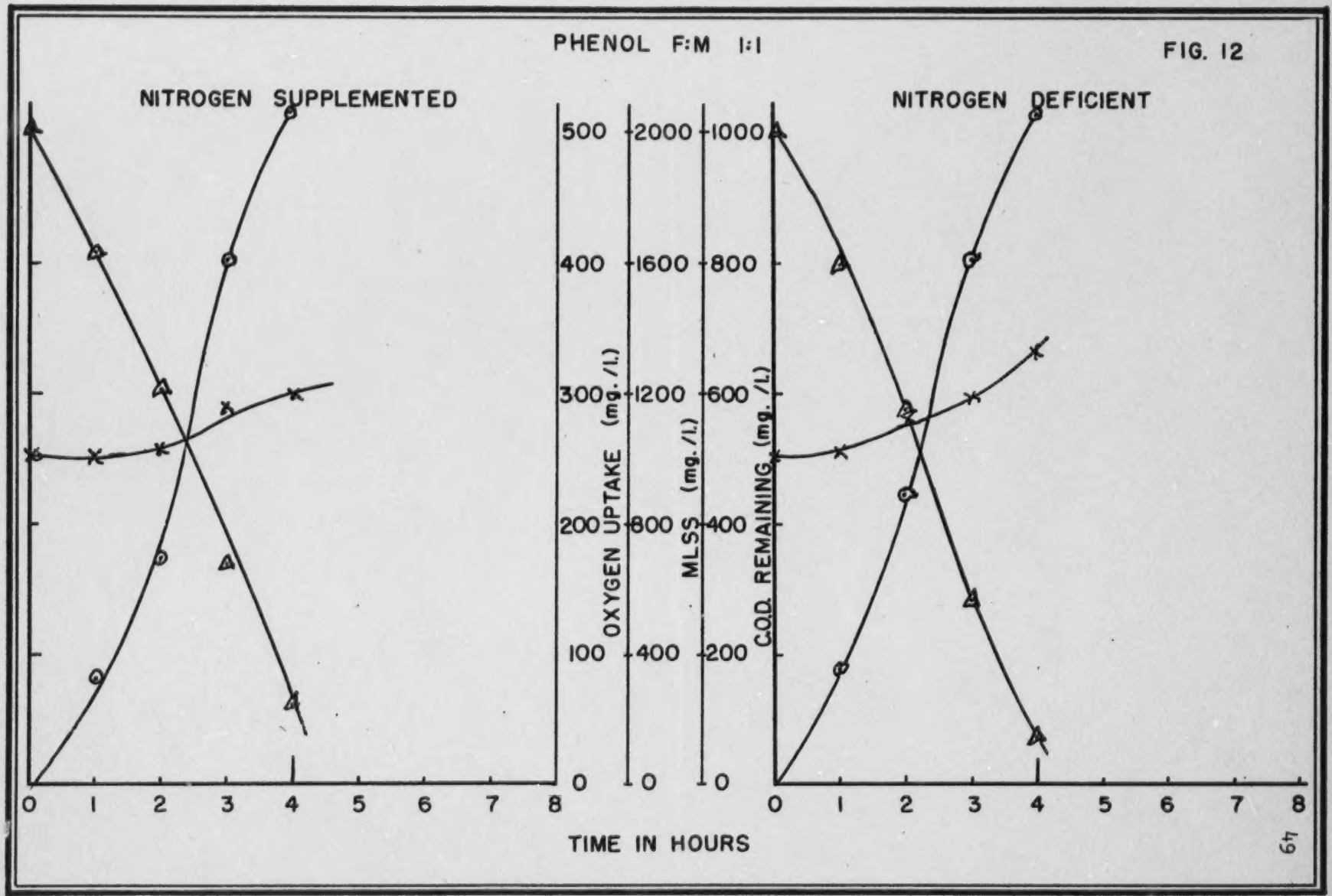

PHENOL F:M 1:1/2
FIG. 7
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
500
400
300
200
100
0
1000
800
600
400
200
0
500
400
300
200
100
0
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./l.)
0 1 2 3 4 5 6 7 8
TIME IN HOURS
44

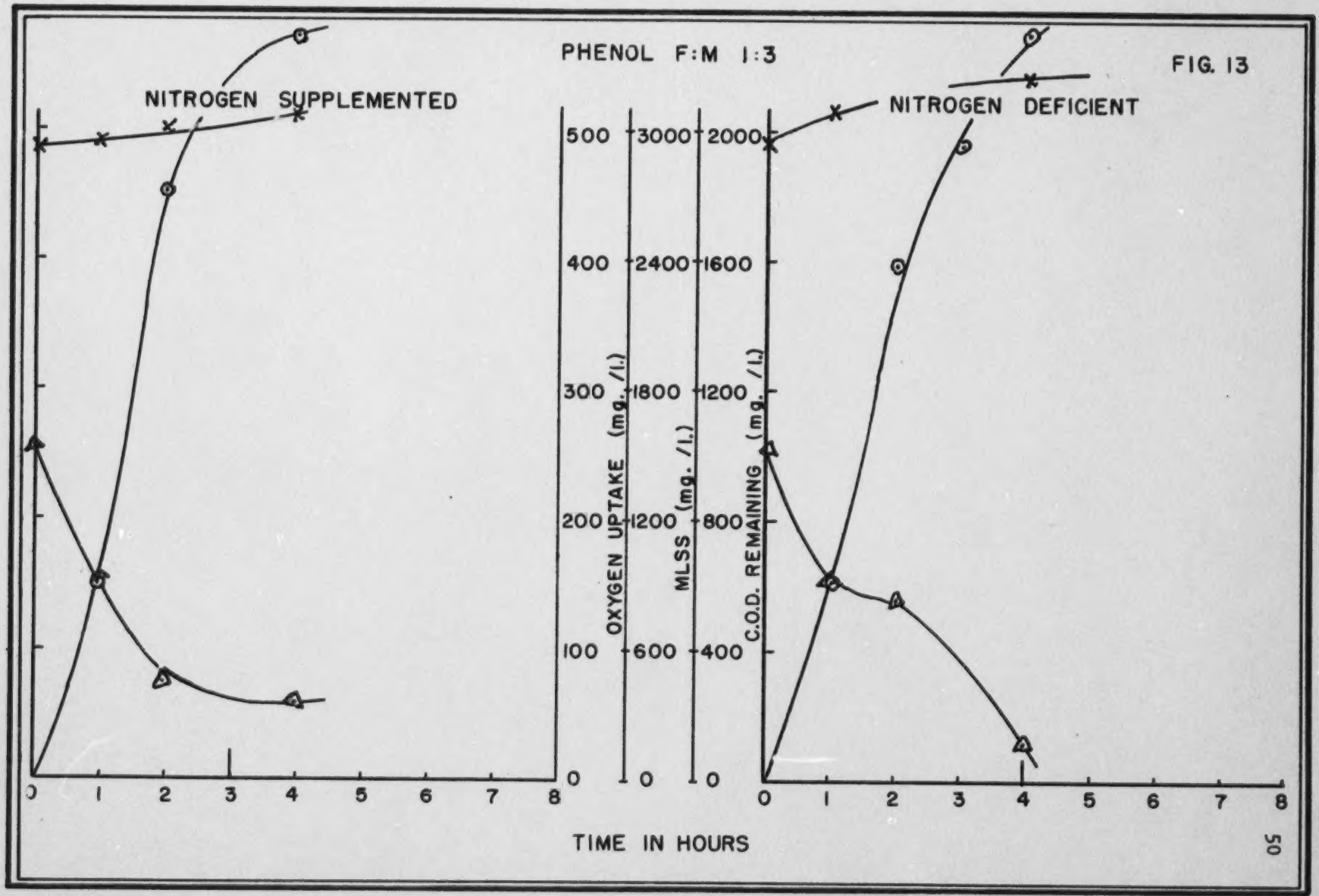

PHENOL F:M 1:1
FIG. 8
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./l.)
500
400
300
200
100
0
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS

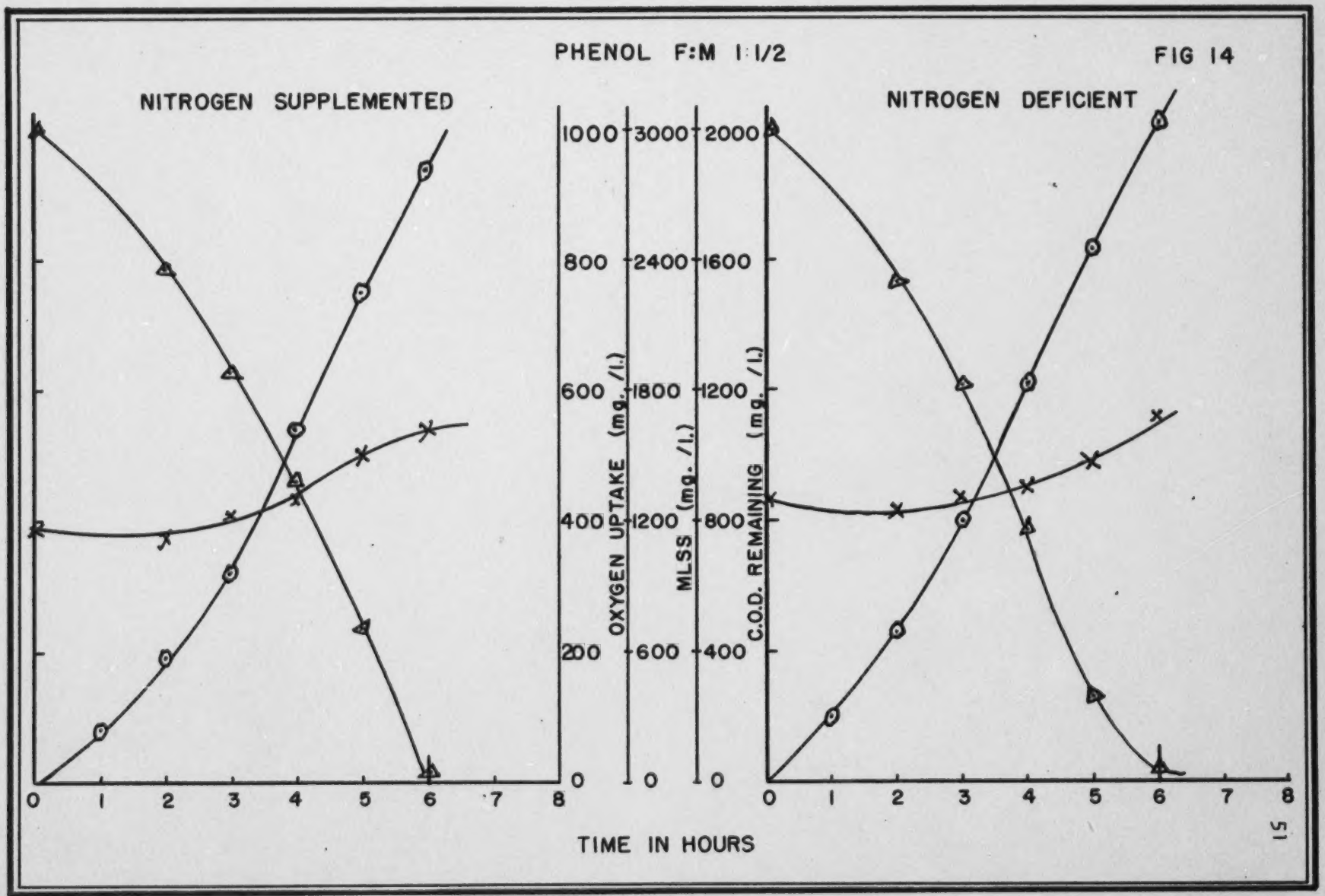

PHENOL F:M 1:2
FIG. 9
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./l.)
500
400
300
200
100
0
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS

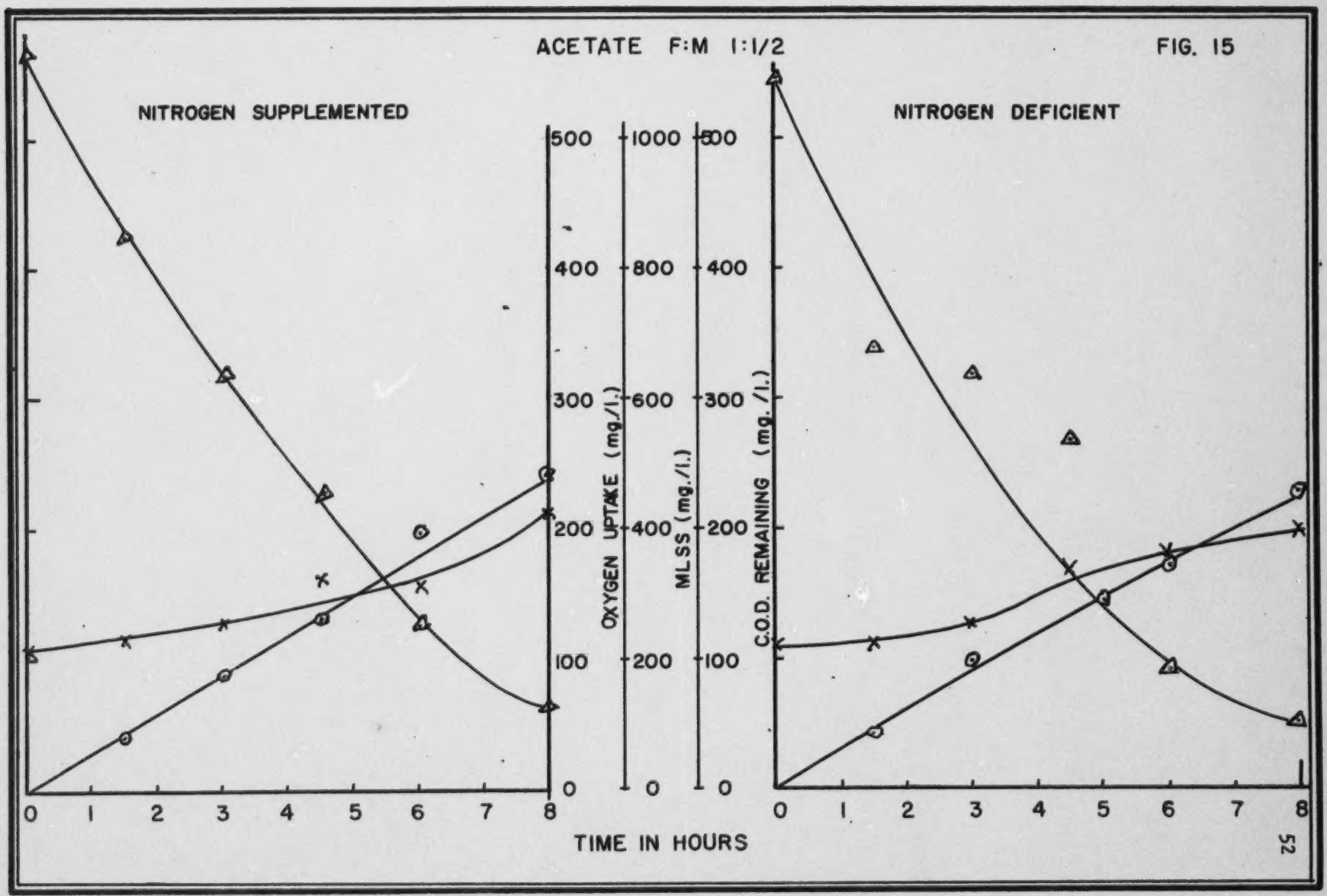

PHENOL F:M 1:1/2
FIG. 10
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
COD. REMAINING (mg./L.)
500
400
300
200
100
0
2000
1600
1200
800
400
0
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS
47

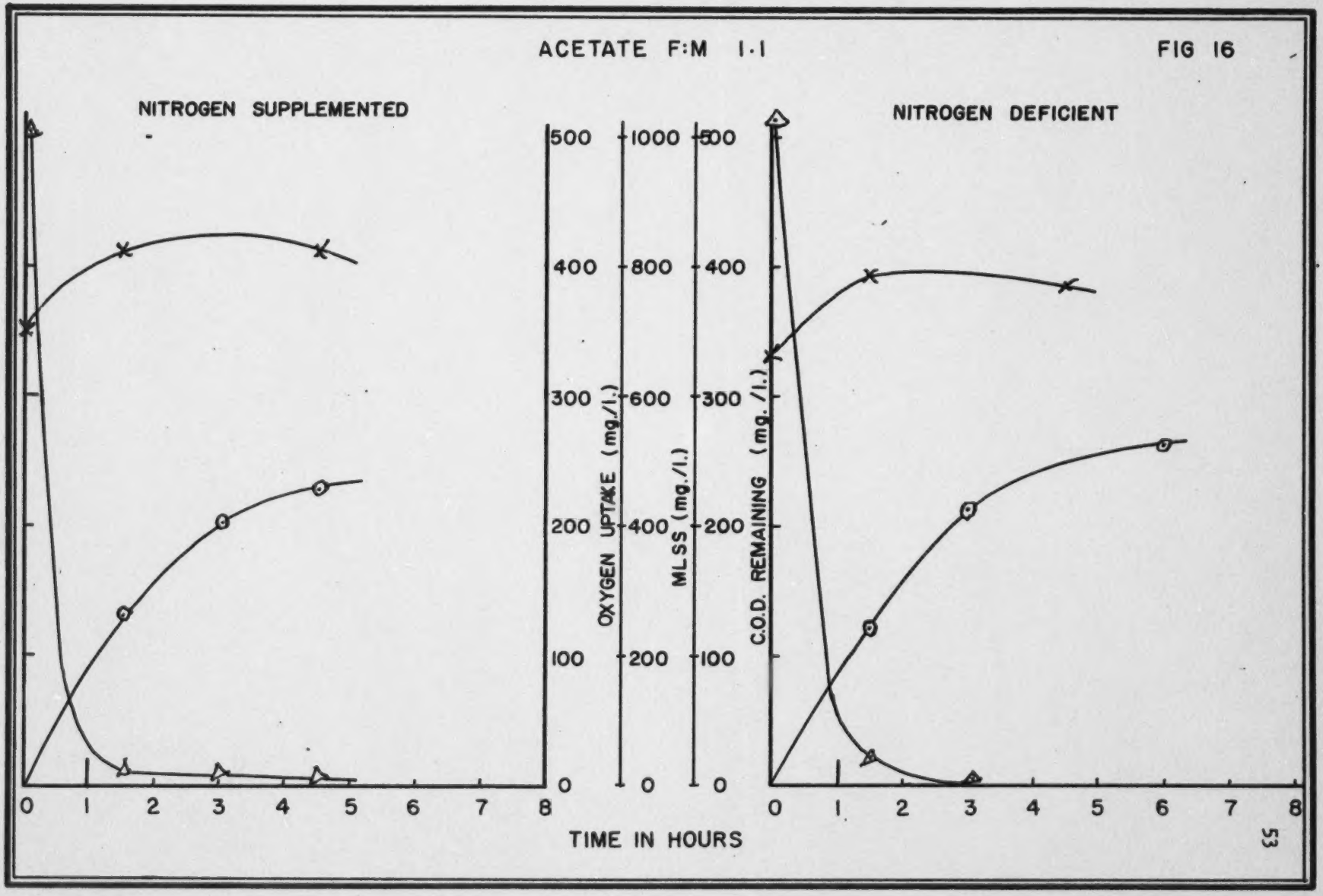

PHENOL F:M 1:1/2
FIG. 11
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./l.)
500
400
300
200
100
0
3000
2400
1800
1200
600
0
2000
1600
1200
800
400
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS
48

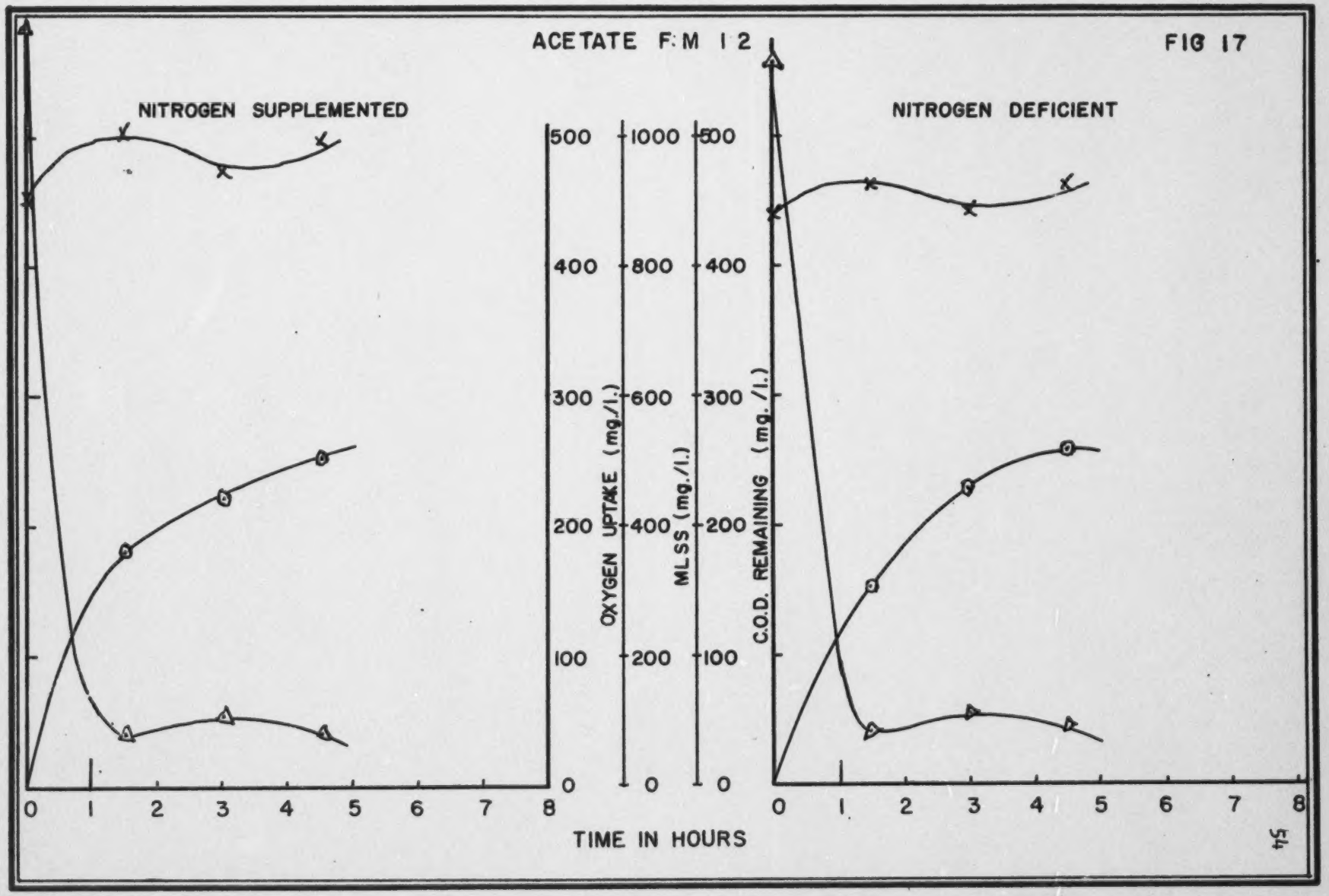

PHENOL F:M 1:1
FIG. 12
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./l.)
500
400
300
200
100
0
2000
1600
1200
800
400
0
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS
49

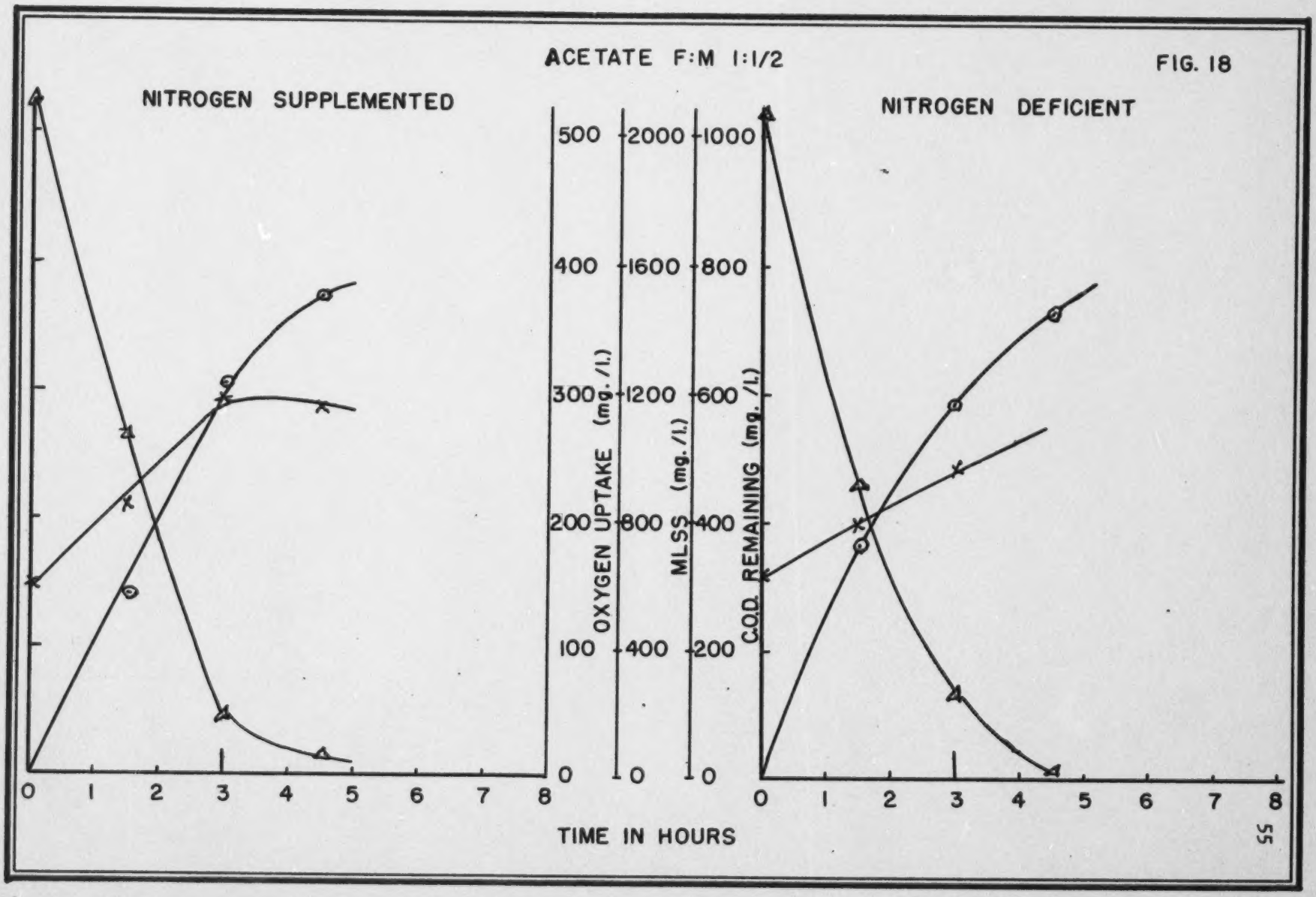

PHENOL F:M 1:3
FIG. 13
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./l.)
500
400
300
200
100
0
3000
2400
1800
1200
600
0
2000
1600
1200
800
400
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS

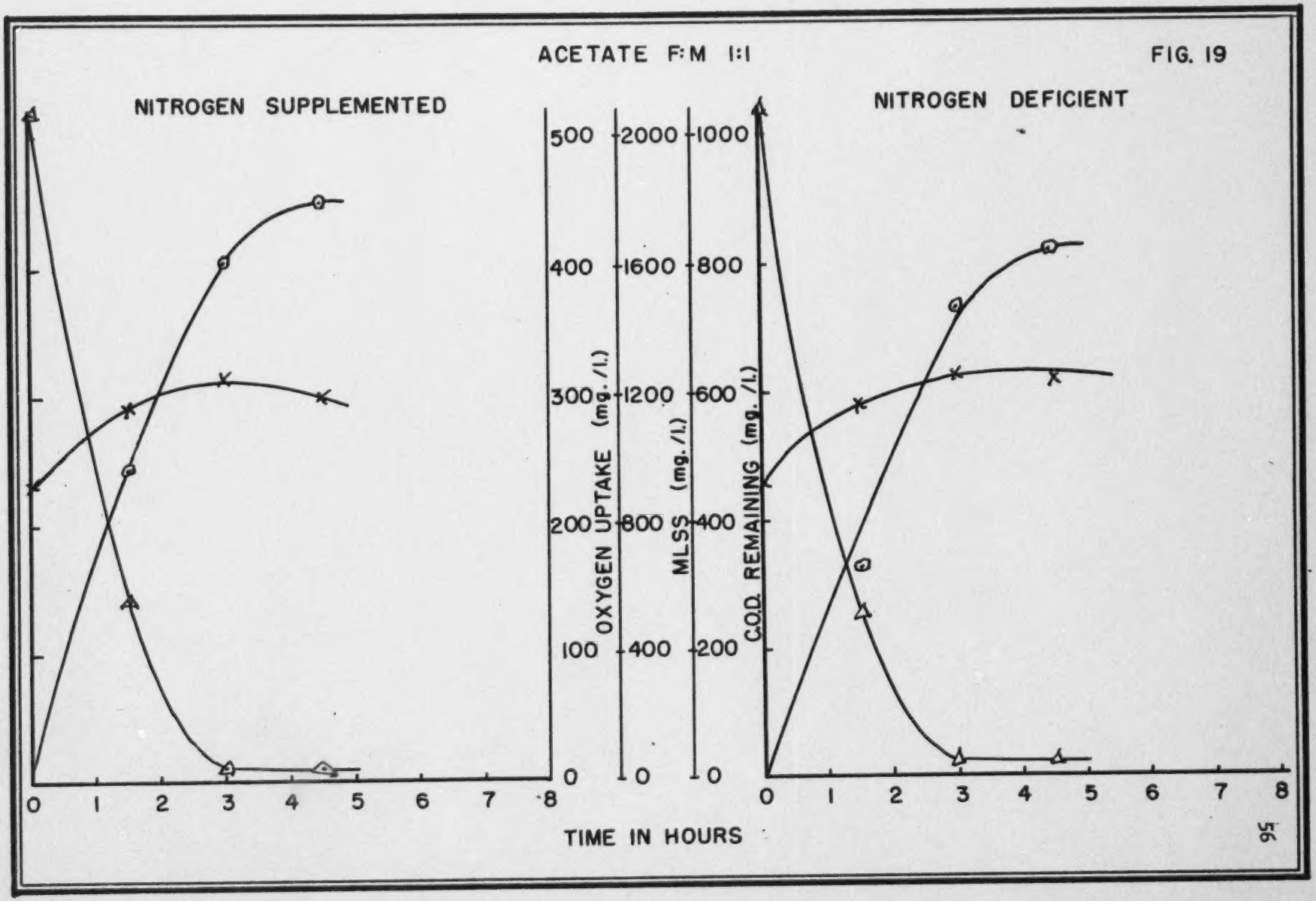

PHENOL F:M 1:1/2
FIG 14
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./l.)
1000
800
600
400
200
0
3000
2400
1800
1200
600
0
2000
1600
1200
800
400
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS

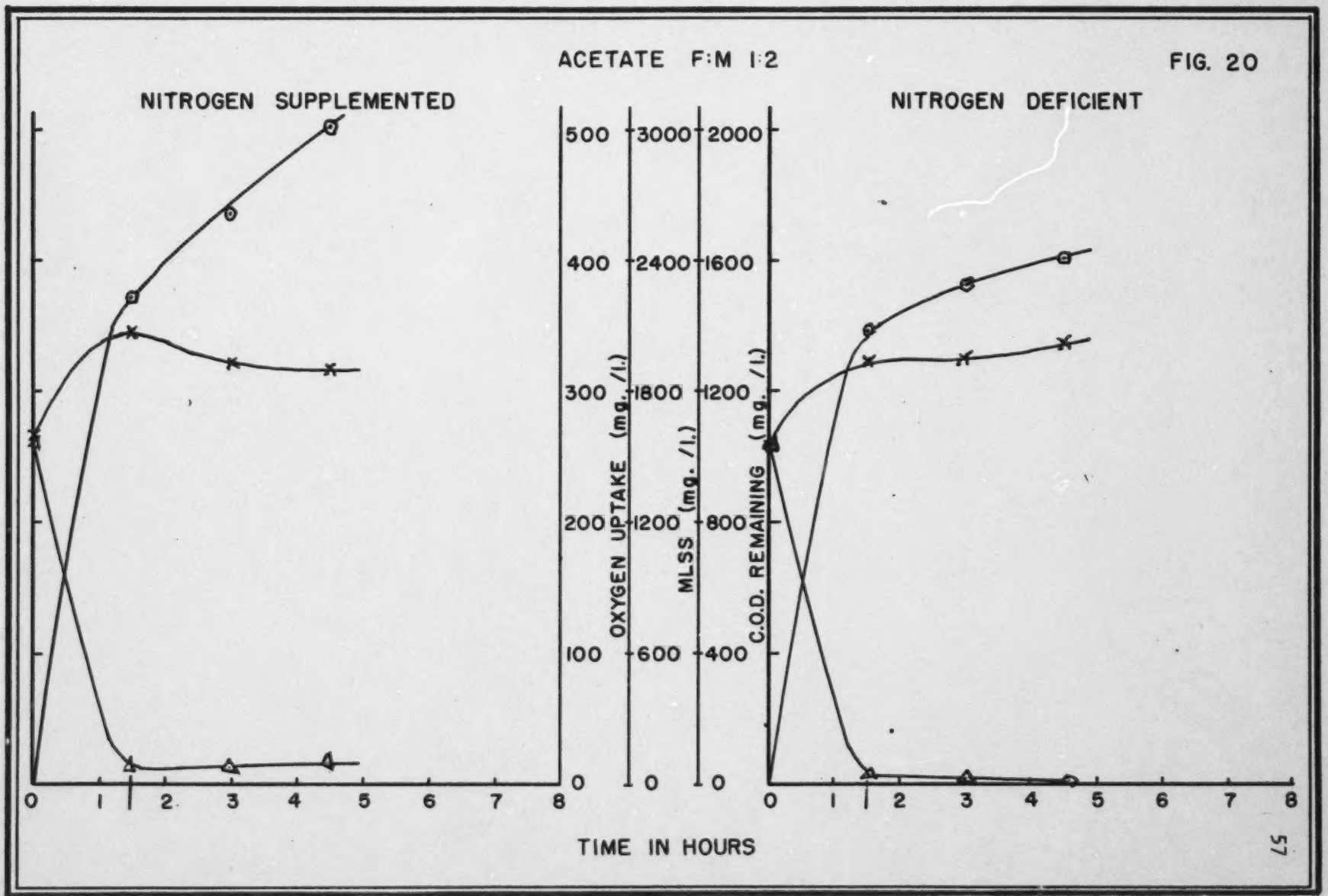

ACETATE F:M 1:1/2
FIG. 15
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./l.)
500
400
300
200
100
0
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS
52

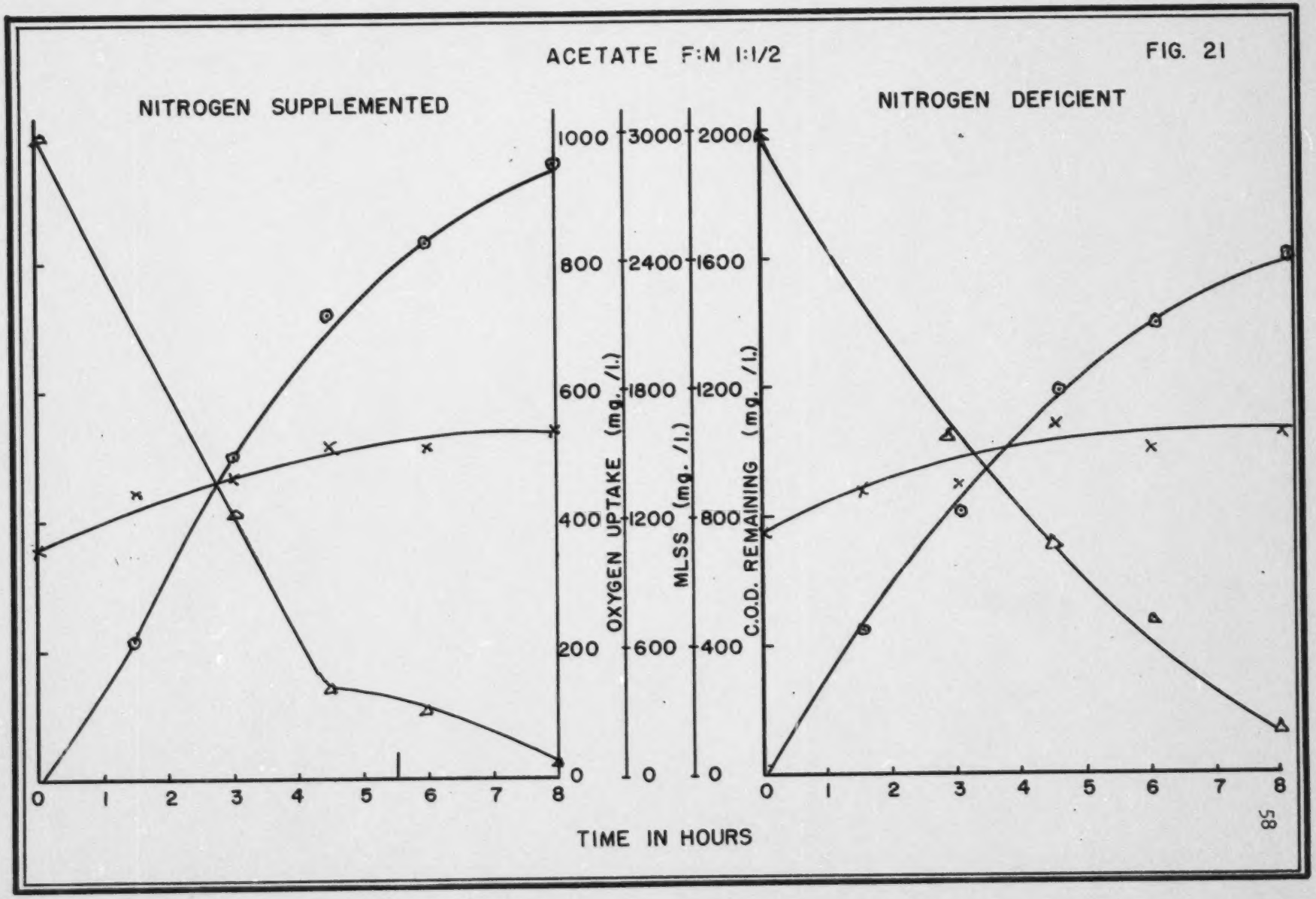

ACETATE F:M 1·1
FIG 16
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./l.)
500
400
300
200
100
0
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS
53

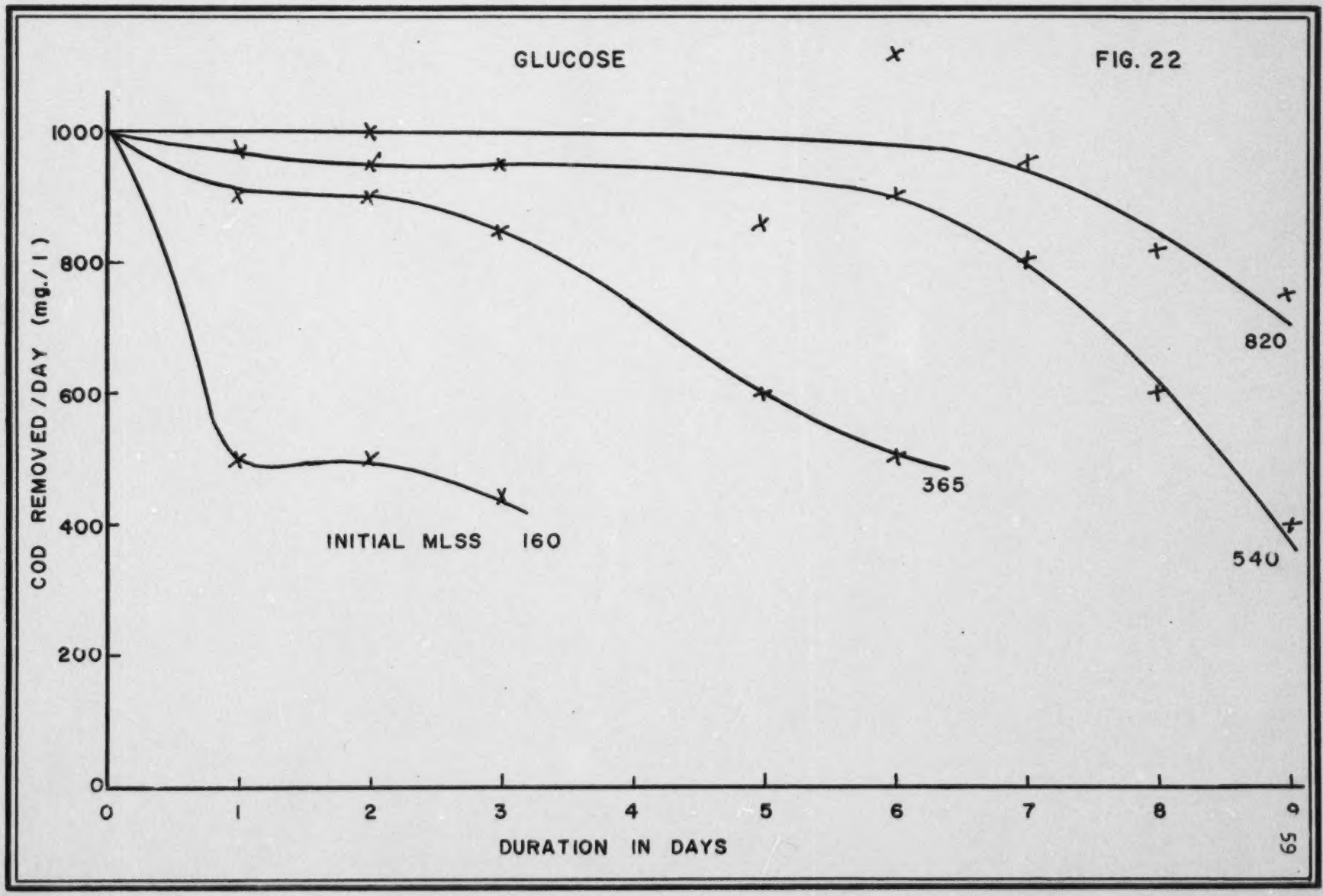

ACETATE F:M 1 2
FIG 17
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./l.)
500
400
300
200
100
0
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS
54

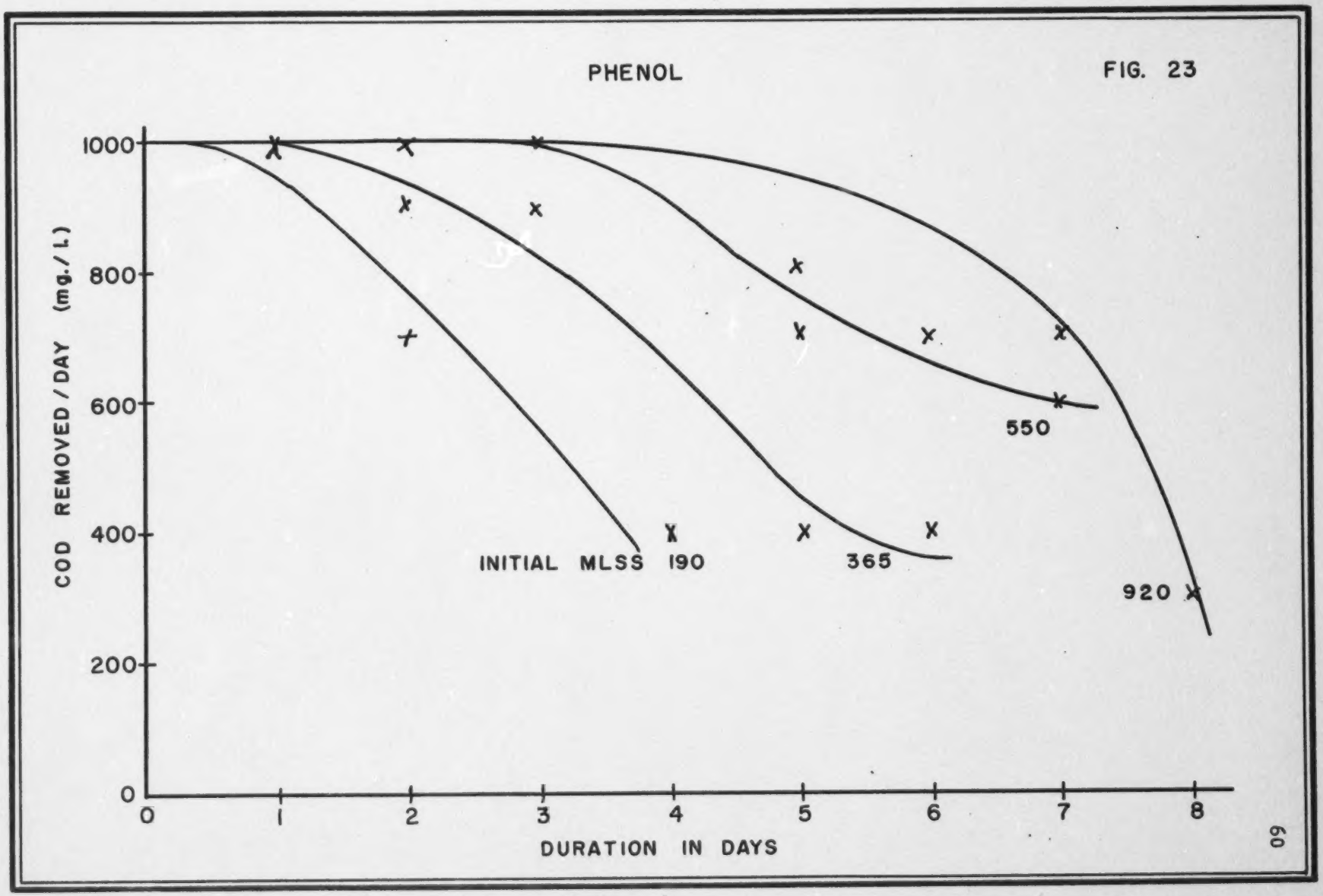

ACETATE F:M 1:1/2
FIG. 18
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
500
400
300
200
100
0
2000
1600
1200
800
400
0
1000
800
600
400
200
0
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./L.)
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 6 7 8
TIME IN HOURS

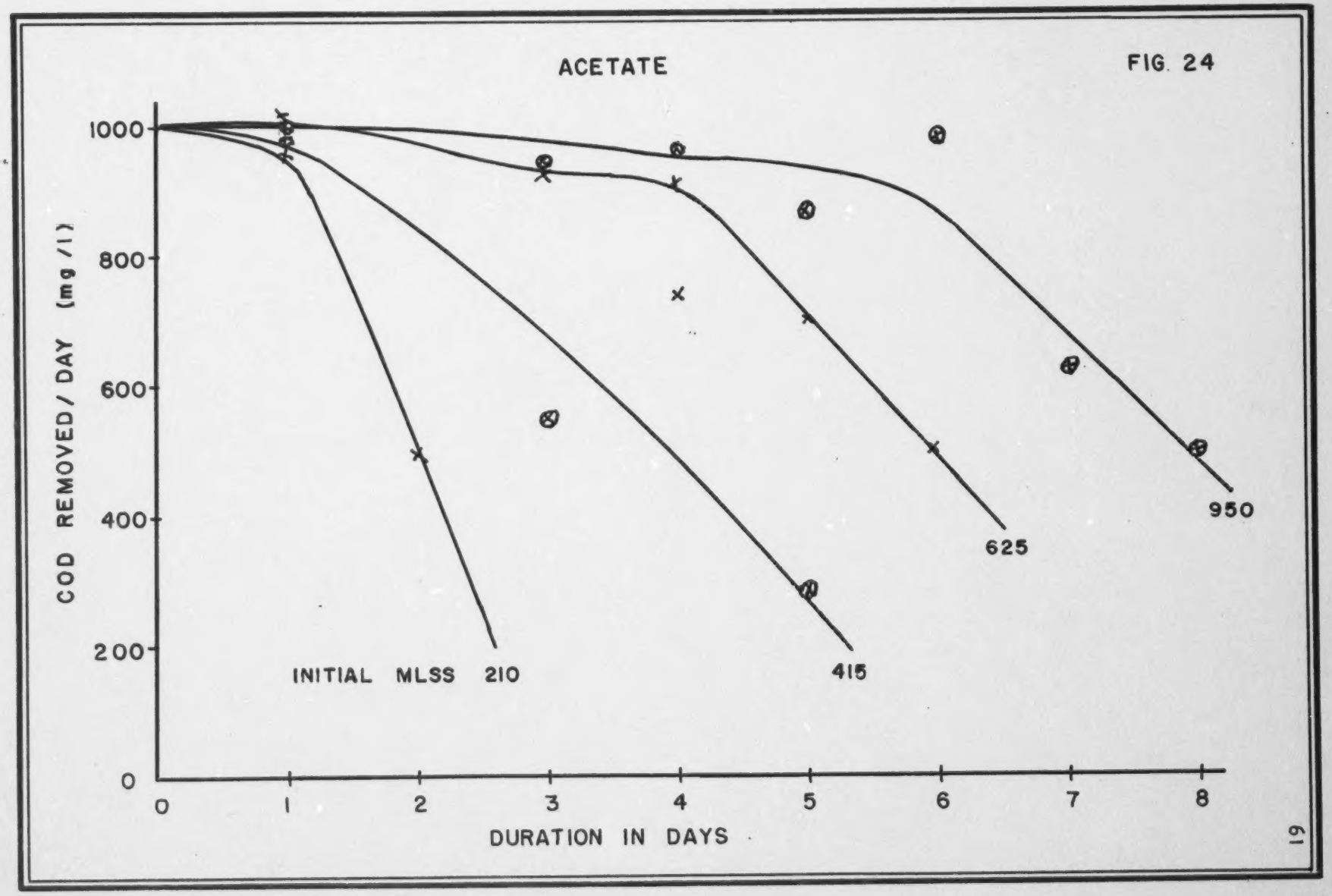

ACETATE F:M 1:1
FIG. 19
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
500
400
300
200
100
0
OXYGEN UPTAKE (mg./l.)
2000
1600
1200
800
400
0
MLSS (mg./l.)
1000
800
600
400
200
0
C.O.D. REMAINING (mg./L.)
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 6 7 8
TIME IN HOURS

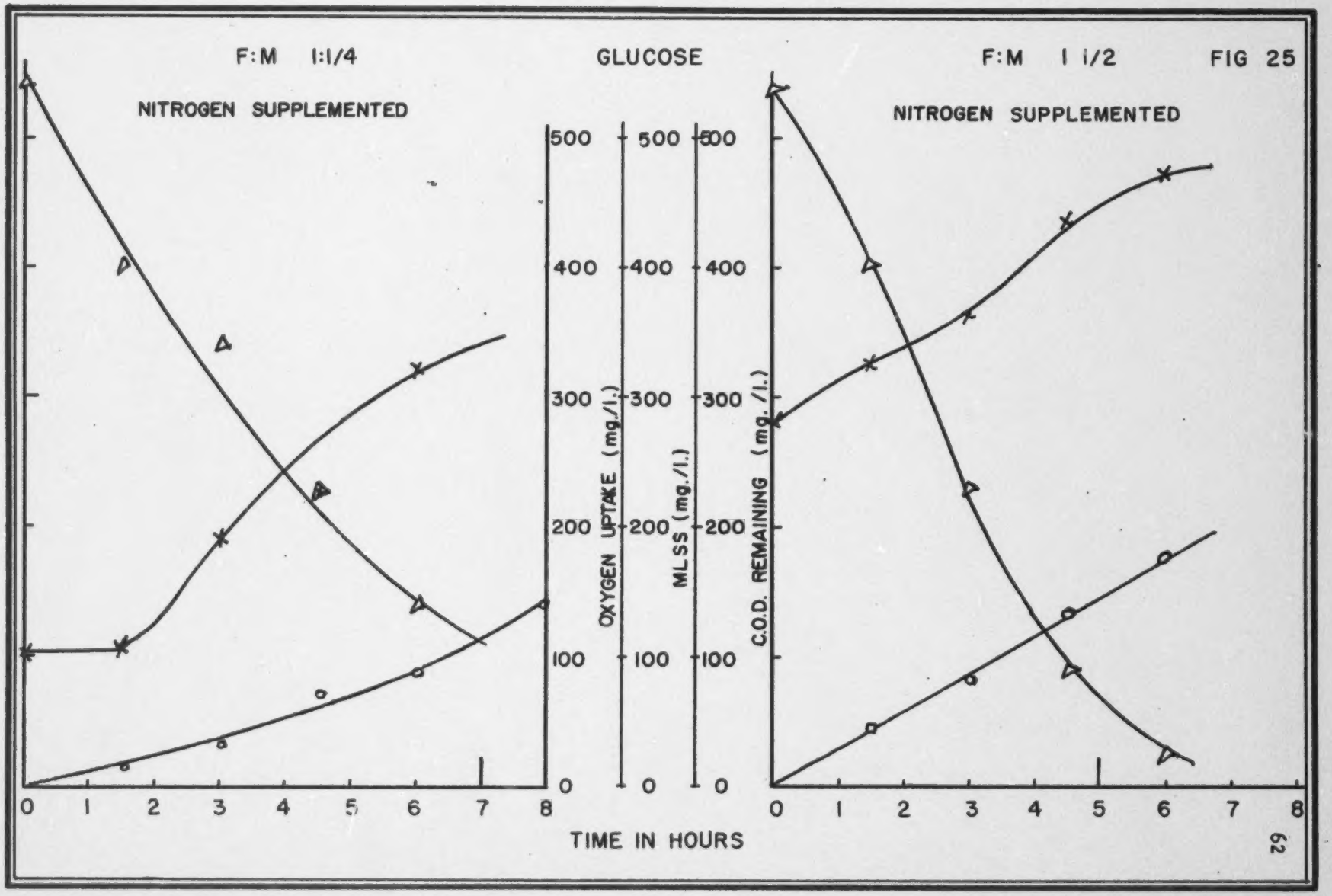

ACETATE F:M 1:2
FIG. 20
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./l.)
500
400
300
200
100
0
3000
2400
1800
1200
600
0
2000
1600
1200
800
400
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS
57

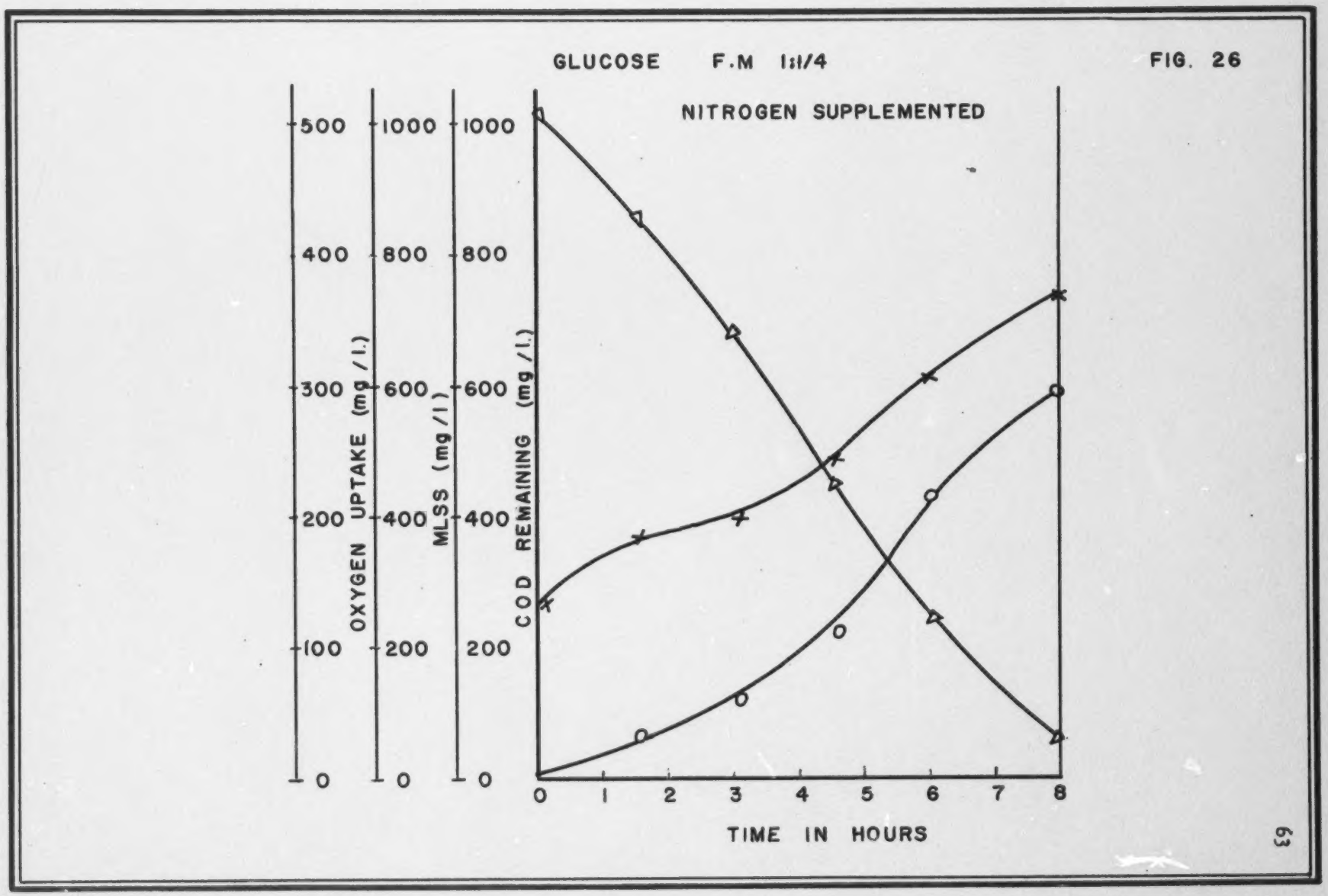

ACETATE F:M 1:1/2
FIG. 21
NITROGEN SUPPLEMENTED
NITROGEN DEFICIENT
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./l.)
1000
800
600
400
200
0
3000
2400
1800
1200
600
0
2000
1600
1200
800
400
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS

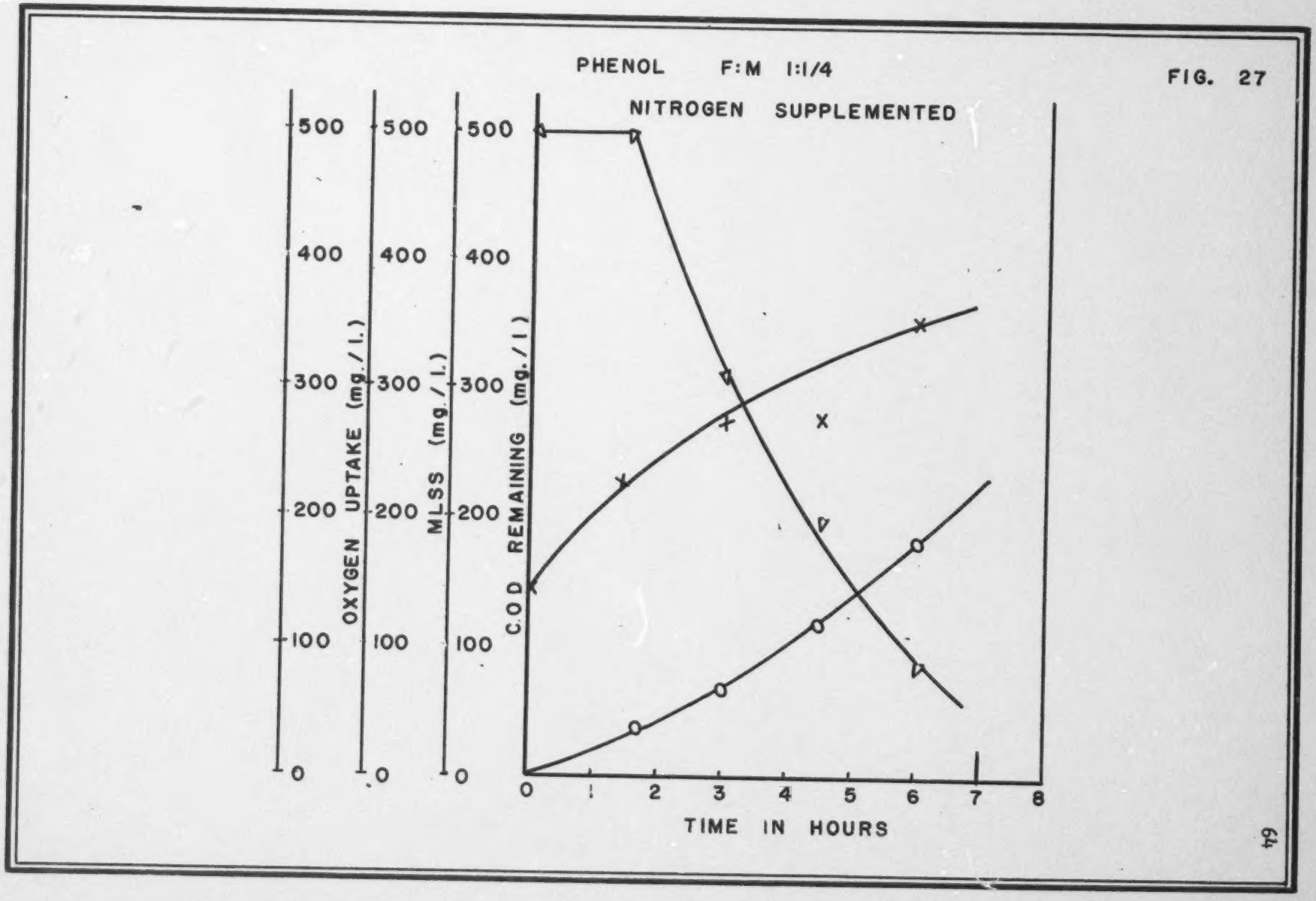

GLUCOSE
FIG. 22
COD REMOVED/DAY (mg./l)
1000
800
600
400
200
0
0
1
2
3
4
5
6
7
8
9
DURATION IN DAYS
820
365
540
INITIAL MLSS 160
59


PHENOL
FIG. 23
1000
800
600
400
200
0
COD REMOVED / DAY (mg./L)
0
1
2
3
4
5
6
7
8
DURATION IN DAYS
INITIAL MLSS 190
365
550
920
60


ACETATE
FIG. 24
COD REMOVED / DAY (mg / l)
1000
800
600
400
200
0
0
1
2
3
4
5
6
7
8
DURATION IN DAYS
INITIAL MLSS 210
415
625
950

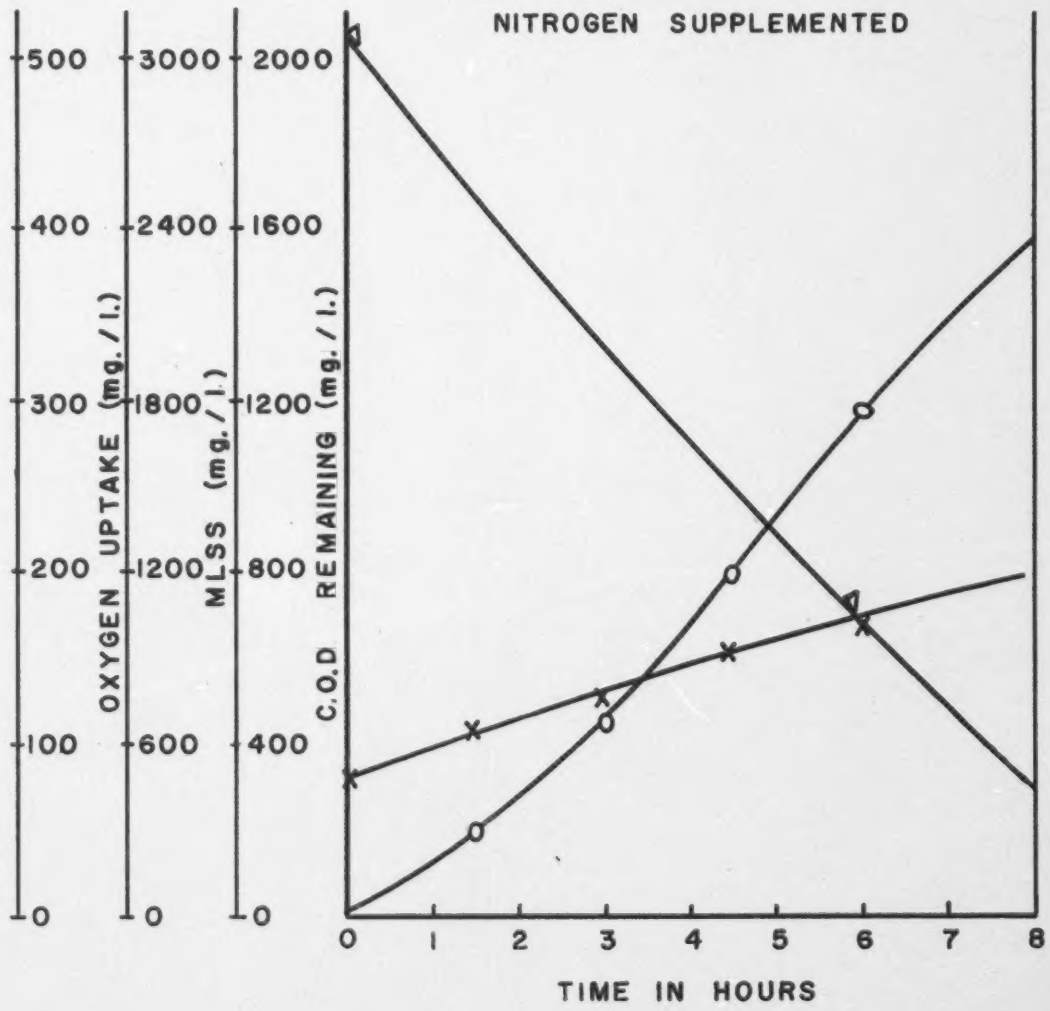

F:M 1:1/4
NITROGEN SUPPLEMENTED
GLUCOSE
F:M 1 1/2
NITROGEN SUPPLEMENTED
FIG 25
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D. REMAINING (mg./l.)
0
100
200
300
400
500
0 1 2 3 4 5 6 7 8
TIME IN HOURS
62


GLUCOSE F.M 1:1/4
FIG. 26
NITROGEN SUPPLEMENTED
OXYGEN UPTAKE (mg/l.)
MLSS (mg/l)
COD REMAINING (mg/l.)
500
400
300
200
100
0
1000
800
600
400
200
0
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8
TIME IN HOURS


PHENOL F:M 1:1/4
NITROGEN SUPPLEMENTED
FIG. 27
500
400
300
200
100
0
OXYGEN UPTAKE (mg./l.)
MLSS (mg./l.)
C.O.D REMAINING (mg./l.)
0 1 2 3 4 5 6 7 8
TIME IN HOURS

ACETATE F:M 1:1/4

FIG. 28



V. DISCUSSION

Sludge Production

It was observed that, within the limitations set for these experiments, the solids production was characteristically higher for the nitrogen deficient systems than for the nitrogen supplemented systems with phenol and glucose, irrespective of substrate concentration. The acetate systems yielded slightly lower solids in the nitrogen deficient units. However, the difference was negligible in all cases, from an engineering viewpoint.

The tables given in the Appendix are all calculated on the basis of 90 percent C.O.D. removal. These values will be used in this discussion. The graphs shown in Figures 1 to 21 are plotted beyond 90 percent C.O.D. removal to show the trend.

Figures 1 to 6 show graphically the solids production with the glucose substrate under the different conditions studied. Figure 4 reveals that with the glucose substrate and an F/M ratio of 1, the increase of solids with a nitrogen deficient system was 66 percent whereas the nitrogen supplemented system had an increase of 63 percent. All other experiments with glucose depicted this trend.

With phenol as the substrate, the same trend was found as noted in Figures 7 through 14. With acetate as the substrate, the solids production was characteristically greater in the nitrogen supplemented systems than in the nitrogen deficient systems as noticed in Figures 15 through 21. The variations were slight but consistent.

As expected, the results show that with higher F/M ratios higher synthesis results, irrespective of the substrate. An example of this is seen in Figure 15, with an acetate substrate of 500 mg/ℓ and an initial

M.L.S.S. of 220 mg/ℓ. Under nitrogen deficient conditions, the net accumulated solids were 170 mg/ℓ. On the other hand Figure 17, with an acetate substrate of 500 mg/ℓ and with an initial M.L.S.S. of 870 mg/ℓ for the same conditions as in Figure 15, shows an accumulated solids of only 35 mg/ℓ. This relationship between the solids production and the F/M ratio was consistent in all Warburg experiments, under both nitrogen supplemented and nitrogen deficient conditions with all three substrates.

For the biological oxidations, the sludge production can be computed from the following considerations. As described earlier a portion of the C.O.D. removed results in the increase of the M.L.S.S. The solids accumulation is governed by the synthesis and the endogenous respiration and can be represented as follows:

$$A = a\,F - b\,M$$

or,

$$\frac{A}{M} = a\,\frac{F}{M} - b \qquad \text{.....(4)}$$

where,

A = increase in M.L.S.S. in lbs/day

F = C.O.D. removed in lbs/day

M = initial M.L.S.S. in lbs

a = portion of the C.O.D. removed that is synthesized

b = mean rate of endogenous respiration expressed as a percent per day

This equation presumes a straight line relationship between the solids accumulation per lb of solids and F/M ratio. This equation implies that when F is zero, A/M is equal to - b, the mean rate of endogenous respiration. This means that when the external food source is absent, there is a decrease in solids mass proportional to the endogenous respiration. As long

as an external substrate is available, it is questionable whether endogenous respiration occurs (3). In an actively metabolizing system such as in those described in this work, b, could be neglected without inducing much error. The usual value for the coefficient, b, is 3 to 5 percent per day. For a study like the one presented here, the value of, b, for an 8 hour period would be less than 2 percent. Considering that the growth equation is only an estimate, this value is negligible. Neglecting the endogenous respiration, the equation for the accumulation of the solids is

$$\frac{A}{M} = a \frac{F}{M} \quad \text{.....(5)}$$

Such an equation is convenient to predict the increase in sludge mass. Figures 29, 30 and 31 show the fractional increase in M.L.S.S. vs C.O.D. loading rate for the substrates glucose, phenol, and acetate, respectively, for nitrogen supplemented and nitrogen deficient systems. The coefficient, a, has a higher value for nitrogen deficient systems for glucose and phenol in comparison with the nitrogen supplemented systems.

Since the coefficient, a, has a lower value for acetate under nitrogen deficient conditions than under nitrogen supplemented conditions, there is a possibility that the end products of metabolism might not be only polysaccharides. Gaudy and Engelbrecht (3) proposed the possibility of lipid synthesis storage from hydrocarbon and fatty acid wastes. This may account for the lower synthesis with acetate under nitrogen deficient conditions. No attempt was made in this study to identify the end products of metabolism.

However, the result of this experiment should not be compared directly with that of Symons and McKinney (17). Their experiment with acetate was conducted under different operational conditions in that the unit was fed daily and operated without wasting for 35 days. Over this prolonged period they had more solids accumulation in the nitrogen deficient system as compared to

the nitrogen supplemented system. In systems supplemented with nitrogen, the protoplasm formed was oxidized more completely during the extensive endogenous period, whereas the polysaccharides formed in the nitrogen deficient system were not oxidized as completely. Hence, there was a build up of the solids in the nitrogen deficient units. In the present study, each system was discontinued as soon as 90 percent C.O.D. was removed so that the effect of endogenous respiration was minimized.

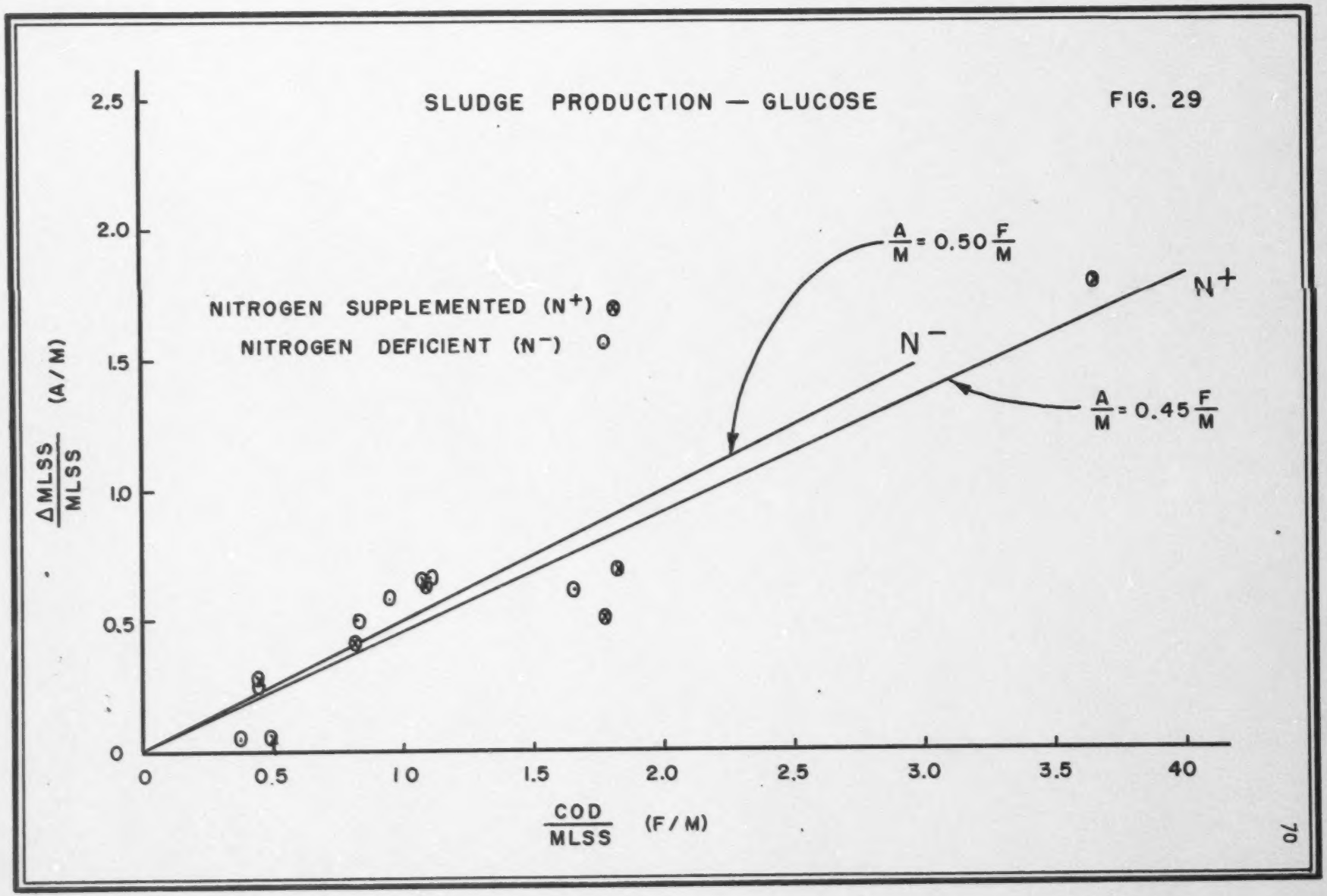
From Figures 29, 30 and 31, the following values were obtained for the coefficient, a, and the units are lbs solids/lbs C.O.D.

	Glucose	Acetate	Phenol
Nitrogen supplemented	0.45	0.42	0.23
Nitrogen deficient	0.50	0.31	0.30

The value of, a, can be used as an index of the biological energy level of the substrate. The magnitude of, a, indicates the comparative energy based on C.O.D. available to the microorganisms. From this study it was observed that glucose yielded the most energy, acetate was second and phenol yielded the least amount of energy. The same conclusions can be reached by comparing the oxygen uptake under identical conditions. The phenol concentration was actually 420 mg/ℓ as phenol in a solution of 1000 mg/ℓ as C.O.D., whereas the weight of glucose was 940 mg for the same C.O.D. On a substrate weight basis, there was slightly more solids production with phenol than with glucose and acetate.

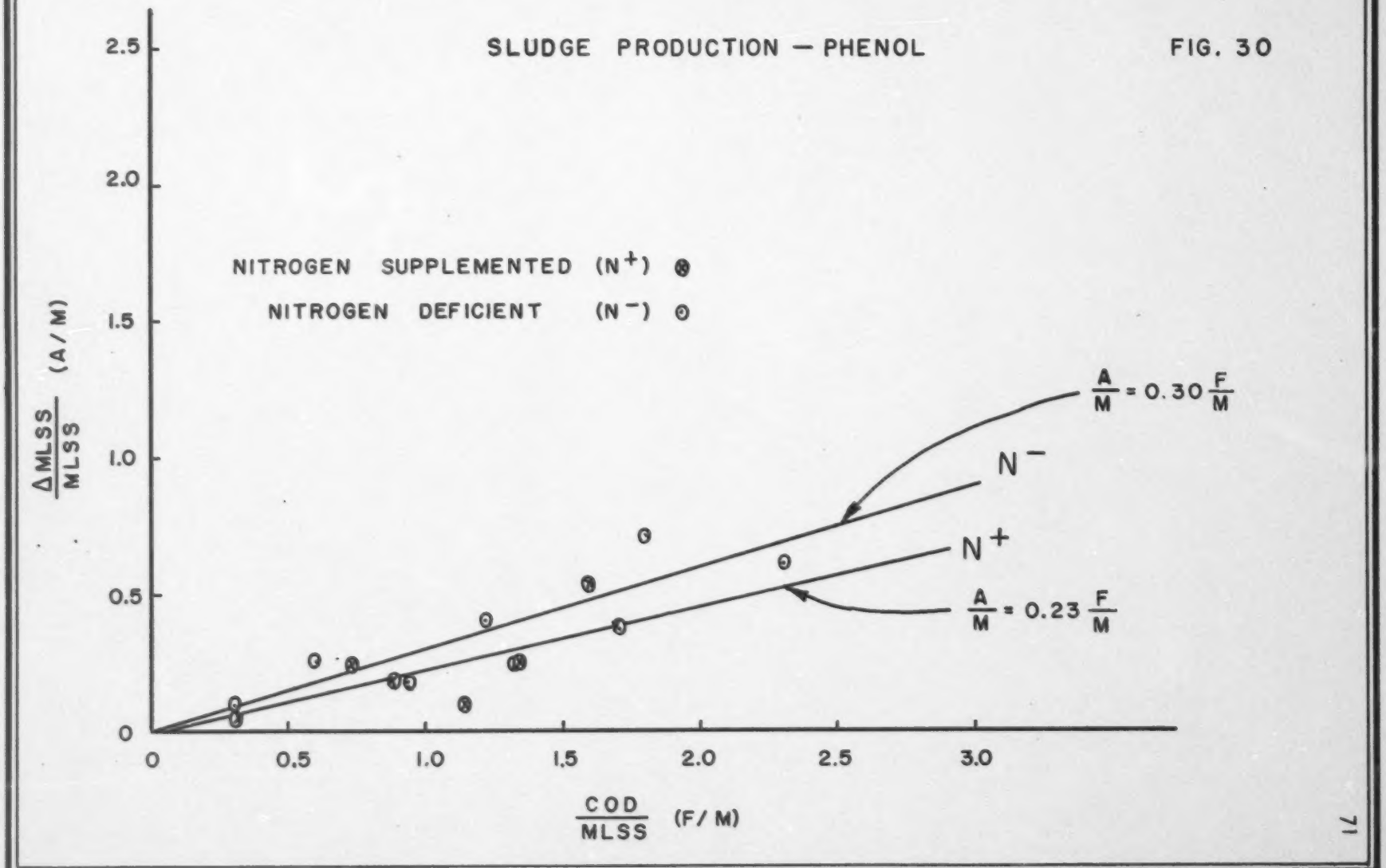
Oxygen Consumption

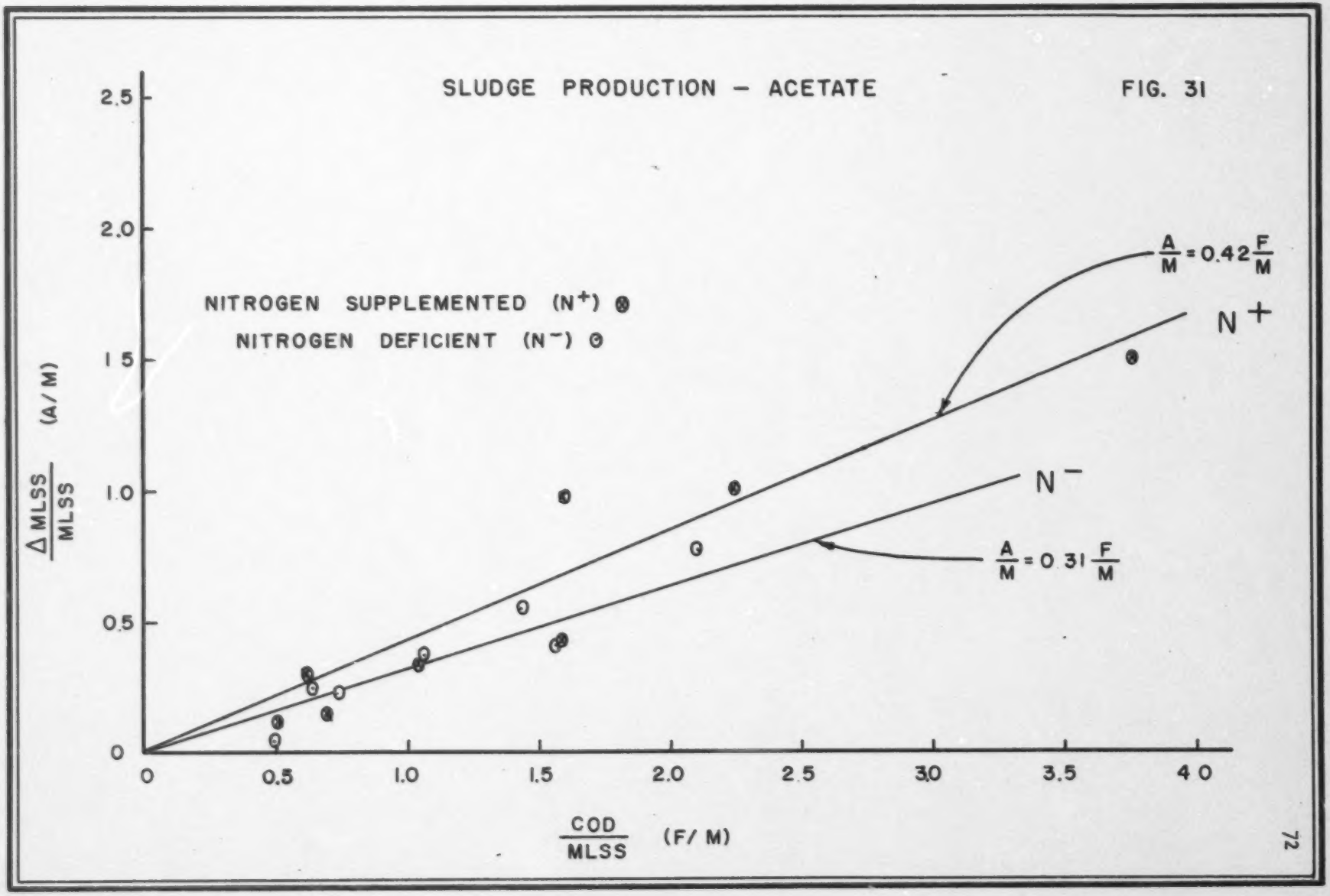
With a few minor exceptions, it is seen from Figures 1 to 21 that with each of the three substrates the nitrogen deficient systems consumed


SLUDGE PRODUCTION — GLUCOSE
FIG. 29
NITROGEN SUPPLEMENTED (N+) ⊗
NITROGEN DEFICIENT (N−) O
A/M = 0.50 F/M
A/M = 0.45 F/M
N+
N−
ΔMLSS/MLSS (A/M)
COD/MLSS (F/M)
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
40
70

SLUDGE PRODUCTION — PHENOL

FIG. 30




SLUDGE PRODUCTION – ACETATE
FIG. 31
NITROGEN SUPPLEMENTED (N+) ⊗
NITROGEN DEFICIENT (N−) ⊙
A/M = 0.42 F/M
N+
N−
A/M = 0.31 F/M
ΔMLSS/MLSS (A/M)
COD/MLSS (F/M)
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
72

slightly less oxygen as compared to the nitrogen supplemented systems. However, the difference in total oxygen uptake was so small that it has little engineering significance.

In this study oxygen utilization per gram of C.O.D. removed was calculated for the interval in which 90 percent C.O.D. reduction occurred. This varied with the substrate concentration and F/M ratio. As the F/M ratio decreased from 5 to 1 the oxygen uptake per gram of C.O.D. removed increased. This trend was seen with all the three substrates. However, with a very low F/M ratio, the oxygen demand per gram of C.O.D. removed was found to be very low.

Based on the F/M ratios, these following different trends were noticed. (a) When the F/M ratio is high, more of the substrate removed resulted in synthesis. A moderate oxygen demand was noted and this demand was exerted for an extended period. An example of this is seen in Figure 27, with phenol substrate of 500 mg/ℓ concentration and F/M ratio of 3. The oxygen uptake per gram of C.O.D. was 485 mg per gram and the average rate of oxygen uptake was 69 mg/hour and this demand lasted for 7 hours. (b) When the F/M ratio is 1 or 2, the oxygen uptake per gram of C.O.D. removed is higher as compared to the previous case. Since a higher number of organisms are present, a higher portion of the substrate removed is oxidized and this exerts a high oxygen demand. A typical example of this situation is shown in Figure 8, with phenol substrate of 500 mg/ℓ concentration and F/M ratio of 1. The oxygen consumed per gram of C.O.D. removed was 650 mg and average rate of oxygen uptake was 93 mg/hour and this demand lasted for 7 hours. (c) At low F/M ratios of 1/2 or 1/3, the organisms absorb more of the substrate on contact only. The phenomenon of absorption became quite significant. Rapid removal of C.O.D. occurred by this process and it is assumed that this was converted to stored food. A case in point is shown in

Figure 9 with phenol substrate of 500 mg/ℓ concentration and an F/M ratio of about 0.5. The oxygen uptake per gram of C.O.D. removed was 415 mg/ℓ. In this case a small amount of total C.O.D. removed is oxidized and more is stored in the cell. Because of a smaller amount being oxidized, a system like this would have a lower oxygen demand than the previous case noted in (b). If such a waste is aerated long enough, the stored food is metabolized, ultimately exerting a high oxygen demand. In fact, it was noticed that the same rate of oxygen uptake continued for 2 to 3 hours after the C.O.D. was depleted. With a nitrogen deficient waste passing through the activated aerator, there is no need to oxidize the stored food since that sludge is not recycled.

The nitrogen deficient systems required longer aeration time as compared to the nitrogen supplemented systems. However, such a difference in time is noticed with high F/M ratios only. Examples of this are seen in Figures 3, 7 and 10. With a phenol substrate concentration of 500 mg/ℓ and initial M.L.S.S. of 350 mg/ℓ as shown in Figure 7, the time taken for 90 percent C.O.D. reduction under nitrogen supplemented condition was 7 hours whereas under nitrogen deficient condition it was 8 hours. Hence the total oxygen uptake noticed would be slightly higher in the latter case. However, the total oxygen uptake would be nearly the same if it is evaluated on the basis of gram of C.O.D. removed.

Rate of Oxygen Uptake

Characteristically with higher M.L.S.S., the oxygen uptake rate was greater but the time taken for metabolizing the substrate was shorter. This trend was the same under both nitrogen supplemented and nitrogen deficient conditions. Table I in the Appendix, shows that with a glucose

substrate of 1000 mg/ℓ concentration, the average oxygen uptake rate per gram of C.O.D. removed varied from 38 mg/ℓ/hour with an initial M.L.S.S. of 265 mg/ℓ to 109 mg/ℓ/hour for an initial M.L.S.S. of 840 mg/ℓ.

The manometers of the Warburg apparatus were read at one or one and half hour intervals. The average rate of oxygen uptake was computed from these readings. In initiating the experiment, Warburg flasks were left open to the atmosphere for 5 minutes in the water bath so as to equilibrate the flask contents to the bath temperature. With high solids, the rate was usually very high during the first few minutes. With a M.L.S.S. concentration of 2134 mg/ℓ for the same 1000 mg/ℓ substrate concentration the oxygen uptake rate was 74 mg/ℓ/hour based on a 1 hour reading. This value was lower than that for the system with 840 mg/ℓ M.L.S.S. It is probable that a high percentage of the 74 mg/ℓ/hour oxygen uptake might have been exerted in the first few minutes and hence the rate would have been much higher. Unfortunately the Warburg manometers were read only once an hour. Hence, the true rate was not accurately determined for those cases in which a very high initial oxygen uptake rate existed for a small fraction of the first hour.

Detention Time vs F/M Ratio

As stated previously, the substrates were metabolized in both the nitrogen deficient and nitrogen supplemented systems. It was observed that the time taken for 90 percent C.O.D. reduction was primarily dependent on the F/M ratio. A higher F/M value required a longer detention time. A low F/M value resulted in rapid removal of substrate but the oxygen rate was very high.

Solids Production with Nitrogen Supplementation

In the conventional activated sludge portion of the scheme under study, biological solids were continuously produced. Since a high F/M ratio encourages synthesis, a low initial concentration of M.L.S.S. was used with a proper nitrogen source supplemented. A comparison of the amount of synthesis can be seen in Figure 25, with glucose substrate of 500 mg/ℓ concentration. An initial M.L.S.S. of 100 mg/ℓ or F/M ratio of 5 resulted in a 270 percent increase in sludge mass with 290 mg of oxygen uptake per gram of C.O.D. removed. On the other hand, an initial M.L.S.S. of 280 mg/ℓ or F/M ratio of 2, produced only 68 percent increase in sludge mass with an increased oxygen uptake equal to 345 mg/gm of C.O.D. removed. The phenol and acetate systems showed similar trends.

In Figure 27, with a phenol substrate of 500 mg/ℓ concentration and F/M ratio of 3.5, it was noticed that the increase in solids was 150 percent. Acetate with a concentration of 2000 mg/ℓ and F/M ratio of 4, as shown in Figure 28, gave rise to 151 percent increase in sludge mass. For the same concentration and F/M ratio, the solids production depended on the energy yield from the substrate. Glucose is a higher energy substrate and hence conducive to greater solids production. A lower energy substrate like phenol yielded lower amounts of solids since a higher fraction of the removed substrate was oxidized to supply the energy needed for the metabolic process.

Considering the whole data presented in these experiments and within the limitations set for the experiments, it is observed that an F/M ratio of 4 is optimum with nitrogen supplementation for protoplasm production. If the initial M.L.S.S. is lower than the above value, the substrate load may exceed the metabolic capacity of the organisms. In such cases, the substrate removal is not accomplished within 6 to 8 hours.

Nitrogen Savings

The process has proven to be effective with all three substrates. Since no nitrogen supplementation is necessary in the portion of the flow treated in the activated aerator, the nitrogen savings are approximately proportional to the flow through the activated aerator. Table VI tabulates the expected savings in the nitrogen cost with each of the three substrates at various concentrations. All the quantities in this table were computed from Tables I to V in the Appendix. The first four columns in Table I deal with the solids production and optimum F/M ratios of the activated sludge portion of the process. The next three columns describe the operational parameters of the activated aerator. The minimum feasible F/M ratio was taken from the existing data. An F/M ratio of 2 seems to be suitable under optimum conditions.

In order to arrive at the proportion of the total flow through the activated aerator the following calculations were made. It is assumed that the waste is homogeneous and of constant C.O.D. The solids accumulated in the conventional activated sludge unit are known. If x fraction of the flow was routed through the conventional unit, the excess solids would be equal to x times Δ M.L.S.S. The remaining fraction of the flow (1-x) passes through the activated aerator and the initial M.L.S.S. or optimum F/M ratio is known. The excess solids from the conventional activated sludge unit should be equal to the required initial M.L.S.S. for the activated aerator. By equating the M.L.S.S. produced, the proportion of the flow through each unit is computed.

$$x \text{ times } \Delta \text{M.L.S.S.} = (1-x) \text{ initial M.L.S.S.} \quad \ldots\ldots\ldots(6)$$

conv. act. sludge activated aerator

The savings in nitrogen cost is dependent on the type of substrate and on

TABLE I

ESTIMATED NITROGEN SAVINGS

Substrate	Conc. mg/ℓ	Protoplasm F/M	Protoplasm Time hrs	Protoplasm Initial MLSS mg/ℓ	Protoplasm Final MLSS mg/ℓ	Protoplasm Excess MLSS mg/ℓ	Activated Aerator Minimum F/M Ratio	Activated Aerator Initial MLSS Needed mg/ℓ	Activated Aerator Time in Hours	% of Flow Through A/S Unit	% Flow Through A/A	Nitrogen Savings
Glucose	500	5	8	100	350	250	1	475	4	66	34	1/3
	1000	4	8	265	740	475	2	543	8	54	46	1/2
	2000	1	5	2050	2885	835	1	2050	6	70	30	3/10
Phenol	500	3	8	146	370	224	1.5	356	8	60	40	4/10
	1000	2	5	594	915	321	2	533	6	62	38	4/10
	2000	2	6	1166	1625	459	2	1294	6	74	26	3/8
Acetate	500	2.5	8	212	425	213	2.5	220	8	50	50	1/2
	1000	2	3	592	1165	573	2	624	3	50	50	1/2
	2000	4	8	1484	1210	726	2	1146	8	60	40	4/10

the fraction of the total flow going through the activated aerator and this varied from 30 to 50 percent.

Waste Sludge

The waste sludge from this process comes from the activated aerator only. The volumes of the sludge can be estimated from the growth equations presented earlier or can be calculated from Figures 29 to 31 for the three substrates. The sludge from the activated aerator is not recycled in the normal course and is wasted from the system requiring further disposal facilities.

Settling Characteristics and Disposal of Sludge

Using membrane filtration, it was observed that the sludge filtering characteristics were poor in the case of nitrogen deficient sludge with glucose substrate only. Hence, a sludge developed predominantly on a carbohydrate type waste may give trouble in vacuum filtration. No difficulty was encountered with the nitrogen deficient sludges developed on acetate and phenol substrates in the process of membrane filtration. These sludges settled in the same time as the nitrogen supplemented sludges. On the whole the settling properties of a nitrogen deficient sludge should be comparable to the nitrogen supplemented sludges.

In this study an attempt was made to determine the degradability of the polysaccharide sludge. One possible method for the disposal of sludge is by the aerobic digestion process. Identical units were set up with return activated sludge from the Urbana-Champaign Sanitary District Treatment Plant. One unit was fed sludge from the glucose nitrogen deficient system and the

was fed waste sludge from the glucose nitrogen supplemented system. Initial results indicated that the nitrogen deficient sludge could be degraded to a degree comparable to the normal sludge. This study was incomplete and, hence, the results have not been included in this work.

Preliminary studies on the disposal of the nitrogen deficient sludge were made using anaerobic digestion. Identical units were set up at 37°C with digesting sludge from the Urbana-Champaign Sanitary District Treatment Plant. Preliminary results showed that the nitrogen deficient sludge was degraded at a rate comparable with the nitrogen supplemented sludge.

Operational Characteristics of the Process

From the conventional activated sludge unit, a large fraction of the return sludge was wasted to promote high synthesis of new cells in that unit. The cells in this system were grown in the presence of excess nitrogen and were young and active.

A study concerning the duration of substrate removal in the absence of an external nitrogen source indicated that the system would continue to function for a length of time proportional to the initial M.L.S.S. The results of this study, with no wasting of solids, are reported in Table IV in the Appendix and shown in Figures 22 to 24 for the three substrates. From Figure 22, it is evident that the glucose system with the higher initial M.L.S.S. (820 mg/ℓ) continued to degrade the substrate at the rate of 1000 mg/ℓ per day for 9 days. The system with an initial M.L.S.S. of 160 mg/ℓ lasted for only 3 days. The same trend was seen in Figures 23 and 24 with phenol and acetate substrates.

This study indicated that in case of emergency when cell production is hindered, the process can still maintain its efficiency. The settled sludge from the activated aerator could be recycled during temporary difficulties. Also, nitrogen could be supplemented at the head end of the activated aeration unit to convert it into a conventional process. Under normal operating conditions, the process is quite stable since no deleterious sludge is recycled.

VI. ENGINEERING SIGNIFICANCE

Treatment of nitrogen deficient wastes by the activated aeration process proposed in this study, has been shown to be practical. Within 8 hours of aeration, 90 percent C.O.D. reduction was obtained with all the three substrates depending on the concentration and F/M ratio.

A high F/M ratio encouraged high synthesis in the nitrogen supplemented and nitrogen deficient units. A ratio of 4 seemed to be the optimum value for the nitrogen supplemented unit. In the activated aerator an F/M ratio of 2 appeared to be optimum for all three substrates. With the above ratios it is expected that the supplemented nitrogen savings would be about 50 percent. Depending upon the nature of the waste, the above optimum ratios are likely to be modified and consequently the proportion of the flow through each of the two units will be modified.

Economy in Oxygen and Nitrogen

For nitrogen deficient waste, if the nitrogen supplied is to be conserved, synthesis must be restricted and oxidation should be increased. This is practiced in the conventional activated sludge plants by maintaining high M.L.S.S. In such cases the loading rates are less than 30 pounds of B.O.D. per 100 pounds of solids per day and the air requirements in these low synthesis plants approach 1200 to 1800 cu. ft. per pound of B.O.D. removed.

On the other hand if the oxygen is to be conserved, synthesis must be encouraged and accordingly more nitrogen has to be supplemented. This is achieved in practice by maintaining low M.L.S.S. in the aeration tank. In these cases, the loading rates are in excess of 30 pounds of B.O.D. per

100 pounds of solids per day and the air requirements in such high synthesis plants vary from 500 to 700 cu. ft. per pound of B.O.D. removed.

The modification presented in this study maintains high synthesis with its accompanying low air requirements as well as low nitrogen supplementation cost.

The detention time in this treatment process is 8 hours or less and this compares favorably with the conventional activated sludge process. This process does not require extra detention volume and hence there would be no increase in the capital outlay. This process can be adopted in the existing activated sludge plants with minor cost since most have two or more aerators and settling tanks.

This method of treatment is likely to produce about 10 percent excess sludge. The sludge settling properties should be comparable with that of a nitrogen supplemented system. Because of a lack of a nitrogen source, an effluent of low fertilizing value would be expected from this process. This is a desirable aspect of an effluent to minimize the plankton blooms in the receiving bodies of water.

This process is flexible in operation and exhibits excellent stability. In case of emergency, the settled sludge from the activated aerator unit could be recycled. The process efficiency is still maintained. It appears that a higher fraction of the flow can be treated in the activated aerator by recirculating part of the settled sludge even under normal conditions.

VII. CONCLUSIONS

From this study, the following conclusions have been drawn.

1. The activated aeration process is feasible and 90 percent C.O.D. removal can be obtained within 8 hours with all three substrates at various F/M ratios.

2. The time taken for C.O.D. removal under nitrogen deficient conditions is comparable to that of a nitrogen supplemented system with F/M ratios less than 2.

3. There is a negligible difference in the total oxygen uptake between the two systems.

4. The oxygen uptake rates differ in both cases with the rate of oxygen uptake being lower with nitrogen deficient systems than with nitrogen supplemented systems.

5. It appears that the activated aeration process is not efficient when the F/M ratio is higher than two.

6. The synthesis of active bacterial cells is optimum with an F/M ratio of 4 under nitrogen supplemented conditions.

7. This process works well and is independent of the strength of the waste within reasonable limits.

8. This system is stable since no deleterious cultures are recycled.

9. The solids production was greater in the case of glucose and phenol under nitrogen deficient conditions whereas with acetate solids production was less under similar conditions.

10. An activated sludge can continue to purify the substrates studied even in the absence of an exogenous nitrogen source for 3 to 8 days depending upon the concentration of the initial M.L.S.S.

VIII. BIBLIOGRAPHY

1. Chasick, A. H., "Activated Aeration at the Wards Island Sewage Treatment Plant," Sewage and Industrial Wastes, 26, 1059 (1954)

2. Eckenfelder, W. W., and O'Connor, D. J., Biological Waste Treatment, Pergaman Press, New York (1961)

3. Gaudy, A. F., Jr., and Engelbrecht, R. S., "Basic Biochemical Considerations During Metabolism in Growing vs Respiring Systems," 3rd Biological Waste Treatment Conference, Manhattan College, New York (1960)

4. Gaudy, A. F., Jr., and Engelbrecht, R. S., "Quantitative and Qualitative Shock Loading of Activated Sludge Systems," Journal of the Water Pollution Control Federation, 33, 805 (1961)

5. Gellman, I., and Heukelekian, H., "Studies of Biochemical Oxidation by Direct Methods, III. Oxidation and Purification of Industrial Wastes by Activated Sludge," Sewage and Industrial Wastes, 25, 1196 (1953)

6. Greenberg, A. E., Gerhard, Klein, and Kaufman, W. J., "Effect of Phosphorus on the Activated Sludge Process," Sewage and Industrial Wastes, 27, 3, 277 (1955)

7. Gunsalus, I. C., and Stanier, R. Y., "The Bacteria Metabolism," Vol. II. Energy Excess: Nutrient Limitation, p. 47-50, Academic Press, New York (1960)

8. Helmers, E. N., Frame, J. D., Greenberg, A. E., and Sawyer, C. N., "Nutritional Requirements in the Biological Stabilization of Industrial Wastes, II. Treatment with Domestic Sewage," Sewage and Industrial Wastes, 23, 884 (1951)

9. Helmers, E. N., Frame, J. D., Greenberg, A. E., and Sawyer, C. N., "Nutritional Requirements in the Biological Stabilization of Industrial Wastes, III. Treatment with Supplementary Nutrients," Sewage and Industrial Wastes, 24, 496 (1952)

10. Holme, T., "Symposium on Polysaccharides," Prague (1958)

11. McKinney, Ross E., Microbiology for Sanitary Engineers, 118, McGraw Hill Publishing Company, New York (1962)

12. Oginsky, E. L., and Umbreit, W. W., "An Introduction to Bacterial Physiology," W. H. Freemen Company, San Francisco (1954)

13. Porges, N., Jasewicz, L., and Hoover, S. R., "Principles of Biological Oxidations," 1-3, Biological Treatment of Sewage and Industrial Wastes, Vol. 1, Aerobic Oxidations, Edited by J. McCabe, and W. W. Eckenfelder, Jr., Reinhold Publishing Corporation, New York (1956)

14. Senez, J. C., and LeGall, J., "Compt. Rend. Acad. Sci.," 250, 404 (1960), Abstract Quoted by Gunsalus and Stanier.

15. Smith, M. W., and Ullrich, A. H., "The Biosorption Process of Sewage and Waste Treatment," Sewage and Industrial Wastes, 23, 1248 (1951)

16. Standard Methods for Examination of Water and Waste Water, 11th Edition, American Water Works Association, (1961)

17. Symons, J. M., and McKinney, R. E., "The Biochemistry of Nitrogen in the Synthesis of Activated Sludge," Sewage and Industrial Wastes, 30, 7, 875 (1958)

18. Torpey, W. N., and Chasick, A. M., "Principles of Activated Sludge Operation," 3-4, Biological Treatment of Sewage and Industrial Wastes, Vol. 1, Aerobic Oxidations, Edited by J. McCabe and W. W. Eckenfelder, Jr., Reinhold Publishing Corporation, New York (1956)

19. Weinberger, L. W., Doctoral Thesis, M.I.T. (1950), Abstract Reported by Sawyer, C. N., 1-1, Biological Treatment of Sewage and Industrial Wastes, Vol. 1, Aerobic Oxidation, Edited by J. McCabe and W. W. Eckenfelder, Jr., Reinhold Publishing Corporation, New York (1956)

20. Weston, R. F., and Eckenfelder, Jr., "Application of Biological Treatment to Industrial Waste, I. Kinetics and Equilibria of Oxidative Treatment," Sewage and Industrial Wastes, 27, 7, 802 (1955)

21. Wilkinson, J. F., Abstract Bacterial Reviews, 22, 58 (1958)

22. Wuhrman, K., "Factors Affecting Efficiency and Solids Production in the Activated Sludge," 1-4, Biological Treatment of Sewage and Industrial Wastes, Vol. 1, Aerobic Oxidation, Edited by J. McCabe and W. W. Eckenfelder, Jr., Reinhold Publishing Corporation, New York (1956)

IX. APPENDIX

TABLE I

WARBURG EXPERIMENTS WITH GLUCOSE

Approx-imate COD/ MLSS	Initial COD mg/ℓ	Final COD mg/ℓ	Δ COD F mg/ℓ	Initial MLSS M mg/ℓ	Final MLSS mg/ℓ	Δ MLSS A mg/ℓ	% Increase in MLSS	Oxygen Uptake mg/ℓ	Oxygen Uptake per gm of COD mg/ℓ	Time Taken hrs	Average Rate of Oxygen Uptake per hr per gm of COD mg/ℓ	A/M	F/M
					N^+ Nitrogen Supplemented								
1	560	85	475	440	725	285	65	150	316	4	79	0.65	1.08
0.5	560	30	530	1072	1125	53	5	115	218	1.5	145	0.05	0.50
2	1140	140	1000	560	840	280	50	230	230	6	38.4	0.50	1.79
1	1100	180	920	840	1370	530	63.2	200	218	2	109	0.63	1.09
0.5	1125	175	950	2134	2710	576	27	70	74	1	74	0.27	0.45
1	1832	182	1650	2050	2885	835	40.6	480	292	5.5	53	0.41	0.81
					N^- Nitrogen Deficient								
1	520	75	445	475	745	270	57	96	216	4	54	0.57	0.94
0.5	540	65	475	1282	1335	53	4.1	70	148	1.5	98.6	0.04	0.37
2	1000	100	900	543	875	332	61	285	316	8	39.5	0.61	1.66
1	1090	190	900	820	1360	540	66	225	250	4	62.5	0.66	1.10
0.5	1075	110	965	2250	2800	550	25	55	57	1	57	0.24	0.43
1	1875	187	1688	2050	3065	1015	50	500	296	6	49.3	0.50	0.82

TABLE II

WARBURG EXPERIMENTS WITH PHENOL

Approx-imate COD/ MLSS	Initial COD mg/ℓ	Final COD mg/ℓ	Δ COD F mg/ℓ	Initial MLSS M mg/ℓ	Final MLSS mg/ℓ	Δ MLSS A mg/ℓ	% Increase in MLSS	Oxygen Uptake mg/ℓ	Oxygen Uptake per gm of COD mg/ℓ	Time Taken hrs	Average Rate of Oxygen Uptake per hr per gm of COD mg/ℓ	A/M	F/M
					N^+ Nitrogen Supplemented								
2	557	87	470	350	435	85	24.3	225	480	7	80	0.24	1.34
1	560	35	525	462	510	48	10.4	340	650	7	93	0.10	1.14
0.5	677	87	590	822	1015	193	23.5	245	415	3	138	0.24	0.72
2	1020	70	950	594	915	321	54	500	510	5	100	0.54	1.60
2	1370	70	1300	486	550	64	13.2	500	365	6	52	0.13	2.68
1	1000	120	880	1000	1190	190	19	575	575	4	144	0.19	0.88
0.5	1110	210	900	2930	3040	110	3.75	570	633	3	211	0.04	0.31
2	2000	30	1970	1166	1625	459	39.4	940	470	6	78.3	0.39	1.69
					N^- Nitrogen Deficient								
2	530	60	470	356	438	82	23	250	474	8	59.3	0.23	1.32
1	560	65	505	415	580	165	39.8	320	571	6	95	0.40	1.22
0.5	554	80	474	786	980	194	24.7	235	425	3	142	0.25	0.60
2	1020	60	960	533	910	377	70.7	510	525	6	85	0.71	1.80
2	1280	200	1080	468	755	287	61.3	400	370	6	52	0.61	2.31
1	1000	70	930	1000	1175	175	17.5	510	510	4	127.5	0.18	0.93
0.5	1040	130	910	2978	3255	277	9.3	575	625	4	138	0.09	0.31
2	2000	30	1970	1294	1675	381	29.4	1010	505	6	84	0.29	1.52

TABLE III

WARBURG EXPERIMENTS WITH ACETATE

Approximate COD/MLSS	Initial COD mg/ℓ	Final COD mg/ℓ	Δ COD F mg/ℓ	Initial MLSS M mg/ℓ	Final MLSS mg/ℓ	Δ MLSS A mg/ℓ	% Increase in MLSS	Oxygen Uptake mg/ℓ	Oxygen Uptake per gm of COD mg/ℓ	Time Taken hrs	Average Rate of Oxygen Uptake per hr per gm of COD mg/ℓ	A/M	F/M
					N^+ Nitrogen Supplemented								
2	560	80	480	212	425	213	100	238	497	8	62	1.0	2.26
1	510	25	485	700	800	100	14.3	95	196	1	196	0.14	0.70
0.5	588	125	463	884	990	106	12	140	302	1	302	0.12	0.52
2	1040	90	950	592	1165	573	97	300	316	3	105	0.97	1.60
1	1030	80	950	907	1230	323	35.6	360	380	2.5	154	0.36	1.05
0.5	1040	40	1000	1600	2075	475	29.6	370	370	1.5	246	0.30	0.62
2	1960	180	1780	1112	1580	468	42	810	455	5.5	82.5	0.42	1.60
					N^- Nitrogen Deficient								
2	540	75	465	220	390	170	77.5	225	485	8	60.6	0.77	2.11
1	505	40	465	630	770	140	22.3	90	193	1	193	0.22	0.74
0.5	552	115	437	870	905	35	4	105	242	1	242	0.04	0.50
2	1030	130	900	624	960	336	54	290	323	3	107.7	0.54	1.44
1	1030	70	960	900	1230	330	36.6	320	334	2.5	133	0.37	1.07
0.5	1030	30	1000	1560	1940	380	24.4	345	345	1.5	230	0.24	0.64
2	1940	140	1800	1146	1600	454	40	800	444	8	55.5	0.40	1.57

TABLE IV

DURATION OF SUBSTRATE REMOVAL WITH NO WASTING OF SLUDGE

Number of days	Feed mg/ℓ	Acetate				Glucose				Phenol			
		MLSS	COD	COD Removed / day	Failure Days	MLSS	COD	COD Removed / day	Failure Days	MLSS	COD	COD / day	Failure Days
0	1000	210	13			160	13			190	4		
1	1000	555	34	980		450	500	490		530	12	992	
2	1000	---	---	500		---	---	500		---	--	700	
3	---	545	>1000	450	3	500	>1000	500	3	590	890	400	
4	1000									490	676	1200	
5	1000									470	>1000	500	5
0	1000	415	0			365	13			365	22		
1	1000	870	35	965		745	105	900		690	70	900	
2	1000	---	---	---		---	---	900		---	---	900	
3	---	795	454	550		1295	350	850		1010	164	900	
4	1000	700	235	765		1325	285	65		865	67	97	
5	1000	---	720	280		1390	720	600		885	690	400	
6	1000	885	>1000		6	1590	>1000	500	6	895	>1000	400	6
7	1000												
0	1000	625	43			540	17			550	22		
1	1000	900	29	1014		855	46	970		835	42	980	
2	1000	---	---	1000		---	---	950		---	---	1000	
3	---	965	101	988		1630	139	950		1150	42	1000	

TABLE IV (Continued)

Number of days	Feed mg/ℓ	Acetate				Glucose				Phenol			
		MLSS	COD	COD Removed / day	Failure Days	MLSS	COD	COD Removed / day	Failure Days	MLSS	COD	COD / day	Failure Days
4	1000	995	97	4		1470	126	7		1100	80	700	
5	1000	930	392	700		1680	392	625		1175	410	700	
6	1000	840	>1000	500	6	1855	290	900		1230	662	700	
7	1000					2340	475	800		1215	>1000	600	7
8	1000					2750	755	600					
9	1000					3050	>1000	400	9				
10	1000												
0	1000	950	0			820	8			920	22		
1	1000	1115	42	960		1240	58	950		1185	34	988	
2	1000	---	---	1000		---	---	1000		---	---	1000	
3	---	1255	84	920		1970	118	940		1435	21	1013	
4	1000	1225	114	970		1590	130	---		1345	118	---	
5	1000	1100	264	850		1960	264	866		1515	316	802	
6	1000	1185	262	998		2370	131	1133		1710	103	1213	
7	1000	1495	675	613		2795	173	958		1850	430	773	
8	1000	1755	>1000	500	8	3030	249	824		2130	>1000	300	8
9	1000					3285	486	763					
10	1000					3300	>1000	400	10				

TABLE V

PROTOPLASM PRODUCTION - WARBURG STUDIES

Substrate	Initial COD mg/ℓ	Final COD mg/ℓ	ΔCOD F mg/ℓ	Initial MLSS M mg/ℓ	Final MLSS mg/ℓ	ΔMLSS A mg/ℓ	% Increase in MLSS	Oxygen Uptake mg/ℓ	Oxygen Consumed per gm of COD mg/ℓ	Time Taken hours	Average Rate of Oxygen Uptake/gm of COD per hour
Glucose	540	50	490	100	370	270	270	142	290	8	36.2
	530	25	510	280	470	190	68	175	345	6	57.5
	1030	60	970	265	740	475	180	295	304	8	38
Phenol	525	25	500	146	370	224	150	270	540	8	67.5
Acetate	2080	260	1820	484	1210	726	151	870	478	9	53